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THE

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A VERTICAL EGG AND FRY INCUBATOR

Roger E. Burrows and David D. Palmer
U. S. Fish and Wildlife Service
Entiat, Washington

THE PRACTICE OF PLACING UNFED FINGERLINGS IN PONDS has eliminated the use of troughs for the first feeding of fingerlings. Without the dual purpose of incubation and rearing, the trough becomes an extremely inefficient tool. As construction costs are based on floor space, the use of inefficient equipment increases these costs unjustifiably.

At the request of the Branch of Game-Fish and Hatcheries, the Salmon-Cultural Laboratory of the U. S. Fish and Wildlife Service, at Entiat, Washington, attacked this problem with the intent of developing more efficient equipment. As a result of these investigations, the laboratory has developed a vertical incubator which will successfully incubate salmon eggs and hold the fry in one-fourth the floor space and with one-fourth the water required for the conventional deep trough.

Design of Equipment

The obvious solution to the problem of more efficient utilization of floor space was to employ the vertical space more efficiently. Troughs, at most, utilize but 16 inches of the available vertical space. The placement of trays one below the other and the direction of the water from the top to the bottom by gravity flow formed the method of approach selected.

Several problems presented themselves: first, the method to be employed for the retention of the eggs and fry; second, the proper water path through the eggs and fry; and third, the amount of water necessary for adequate maintenance during the various stages of incubation. Each of these problems required much preliminary experimentation. In the interest of brevity, these preliminary experiments will not be reported. It will suffice to say that the problem was first attacked in 1950 and the final design was not completed until 1954.

Use of a removable basket within a metal tray proved to be the most practicable method for the retention of the eggs and fry. The removable basket allowed for easy stocking and cleaning and the eventual removal of the fish. The tray maintained the water level and directed the water flow. A woven plastic-screen bottom and perforated aluminum plate at one end of the basket retained the eggs and fry yet allowed circulation of water. This type of equipment was first developed in Europe as an adaptation of what has been described by Nordqvist (1893) as the "Californian apparatus." A further modification as described by Schaeperclaus (1933) more nearly resembled the basket and tray developed for the vertical incubator. Although each tray and basket in the European equipment formed a self-contained unit and the water flowed from one tray to the next, they were assembled in steps, one below the other; so this arrangement saved no more floor space than the conventional Clark-Williamson hatching trough. A further development along these lines was the drip incubator, described by Simon and Roberts (1941).

To be practicable, a vertical incubator must retain the fry until the feeding stage; otherwise, no saving in floor space would

be achieved. The path of the water flow through the incubation basket proved to be the prime factor in the retention of fry. Water was introduced at one end of the tray, was forced under the basket and up through the plastic-screen bottom, and then was allowed to flow through the perforated metal plate at the end of the basket and over the lip of the tray into the tray below. The water upwelling through the bottom screen proved very effective in preventing the smothering of fry. This type of water path was first developed in the Williamson trough and has been generally adopted for the retention of fry.

The number of times the water is reused, the number of eggs and fry per basket, and, indirectly, the efficiency of the equipment would be dependent upon the maximum oxygen demand during the incubation period and upon the intermediate aeration accomplished by the spill of water between trays. Tests indicated that the critical period of oxygen demand was just prior to the "swim up" of the fry. At this stage slightly more than 3 parts per million of oxygen was necessary for the adequate maintenance of the fry in this equipment. Water entering at the rate of 2/3 gallon per minute and containing 7 parts per million of oxygen when introduced would supply 7 trays containing a total of 56,000 chinook fry before suffocation occurred. A total of 1-1/2 gallons per minute of water maintained 15 trays, or 120,000 chinook fry, at an oxygen content of 7 parts per million throughout the series. The maintenance of the oxygen supply may be explained by the more efficient aeration attained between trays by the increased flow. In actual operation, a flow of 2 gallons per minute is preferable to avoid short-circuiting the flow pattern within the baskets and because it gives a smoother fall of water between trays.

This type of incubator is readily adaptable to production operations. To conserve space, 10 vertical banks of 15 trays each are included in 1 frame or battery and 2 batteries are placed back to back with a space between them to allow for cleaning of the basket screens. A perspective drawing of a production incubator is shown in figure 1. Such a double-battery incubator has a capacity ranging from 2,000,000 of the largest chinook salmon eggs to 6,000,000 blueback salmon or trout eggs.

The picking trough that is shown in figure 2 is designed to hold the incubator baskets and is used during the distribution of eggs into the baskets and during the cleaning of the eggs and fry. The well at the lower end of the trough is for the retention of the eggs during measurement and distribution into the baskets.

An incubation room of this type containing 5 double batteries, picking troughs, and adequate aisle space would require a floor space of 20 feet by 40 feet. Such a room would have a capacity of 10,000,000 chinook salmon eggs or 30,000,000 blueback salmon or trout eggs.

Construction

The details of construction for the baskets and trays are shown in figures 3 and 4, and the details of the frame construction in figure 1. Certain phases of the construction are critical to successful operation and bear emphasis.

The flow of water between trays is controlled and directed by the angle of the tray lip, the sharp bottom edge of the tray, and the level supporting frame. The angle of the tray lip may require adjustment to assure a smooth-flowing stream of water between one tray and the next. Ideally, the flow should be free falling, but sometimes water will run down the end of the tray. If the bottom edge of the tray is not a sharp 90° angle, the water will tend to follow under the bottom of the tray and drip directly into the basket below, thus short-circuiting the water path. The supporting frame for the trays must be level if the proper water level is to be maintained within the basket and if the fall between trays is to be uniform.

The fall of water between trays introduces air bubbles underneath the bottom screen of the basket. To allow for the escape of these bubbles and to prevent the formation of air pockets beneath the

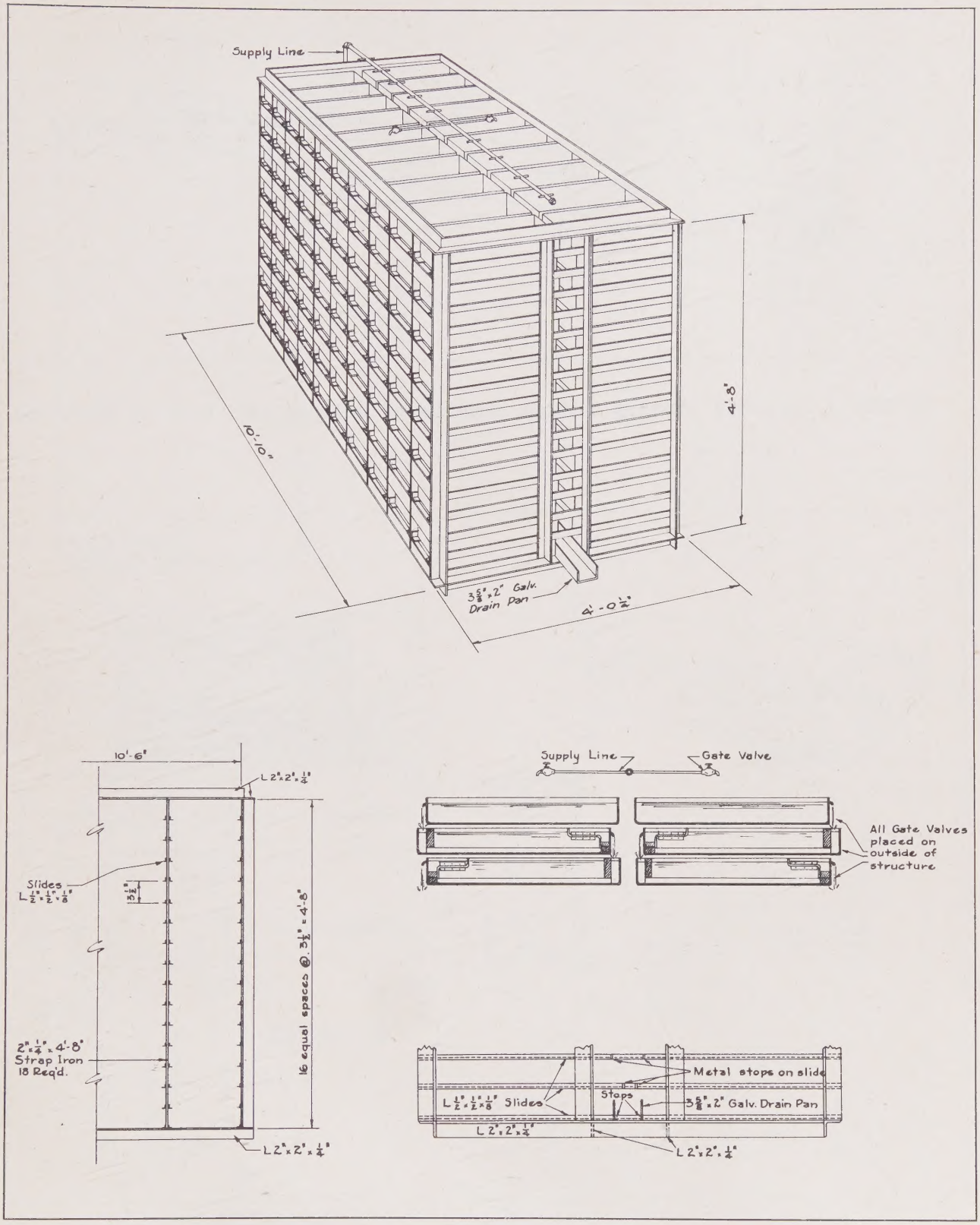
Supply Line
10'-10"
4'-8"
3⅝" x 2" Galv. Drain Pan
4'-0½"
10'-6"
L 2" x 2" x ¼"
Slides L ½" x ½" x ⅛"
2" x ¼" x 4'-8" Strap Iron 18 Req'd.
16 equal spaces @ 3½" = 4'-8"
L 2" x 2" x ¼"
Supply Line
Gate Valve
All Gate Valves placed on outside of structure
Metal stops on slide
Stops
L ½" x ½" x ⅛" Slides
3⅝" x 2" Galv. Drain Pan
L 2" x 2" x ¼"
L 2" x 2" x ¼"

FIGURE 1.--Incubator and details of water path and framework construction.

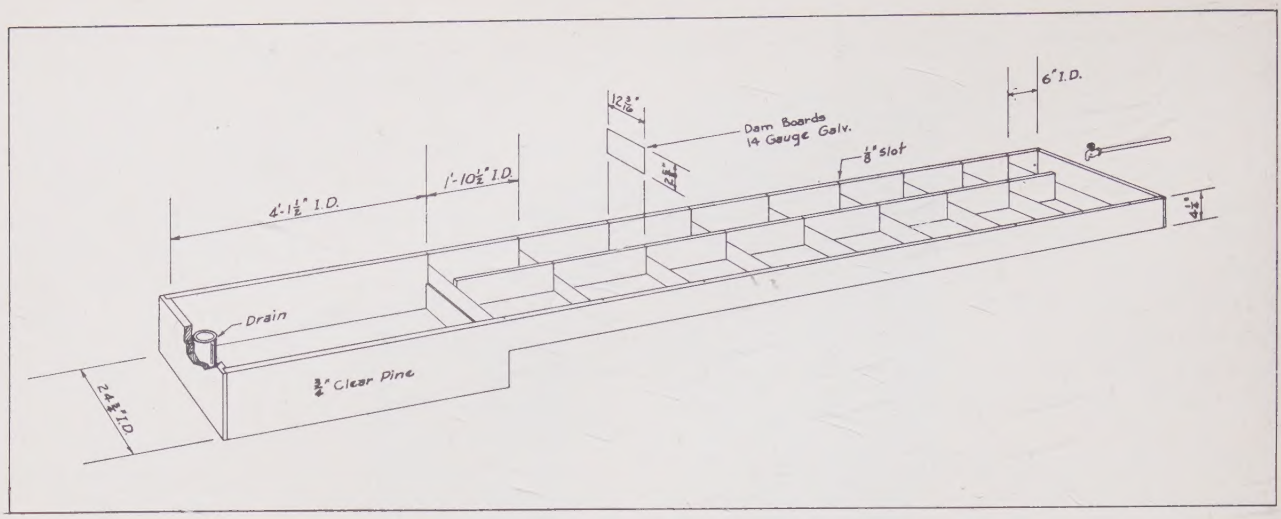

FIGURE 2.--Double picking trough capable of holding one bank of baskets.

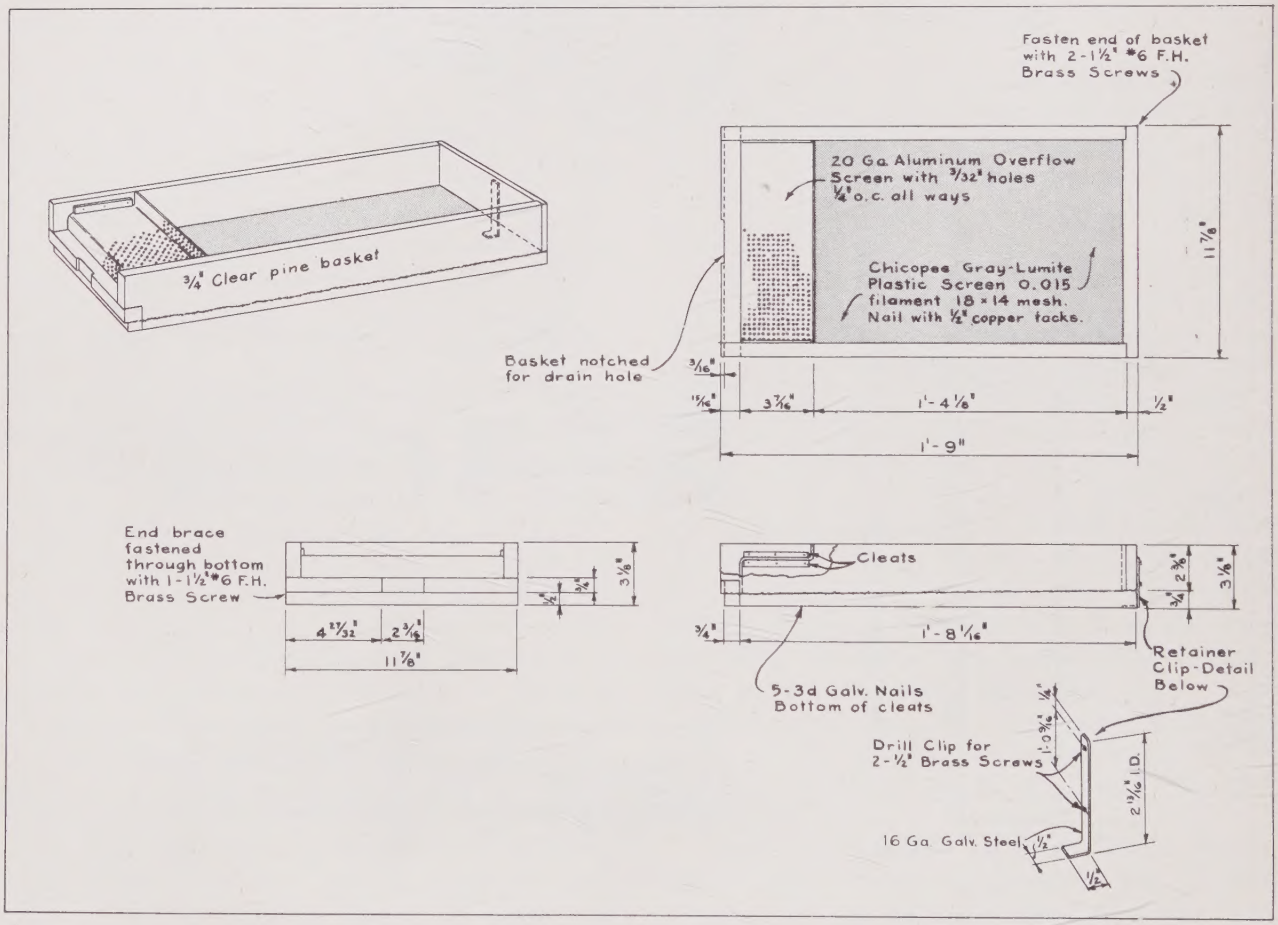

FIGURE 3.--The incubator basket.

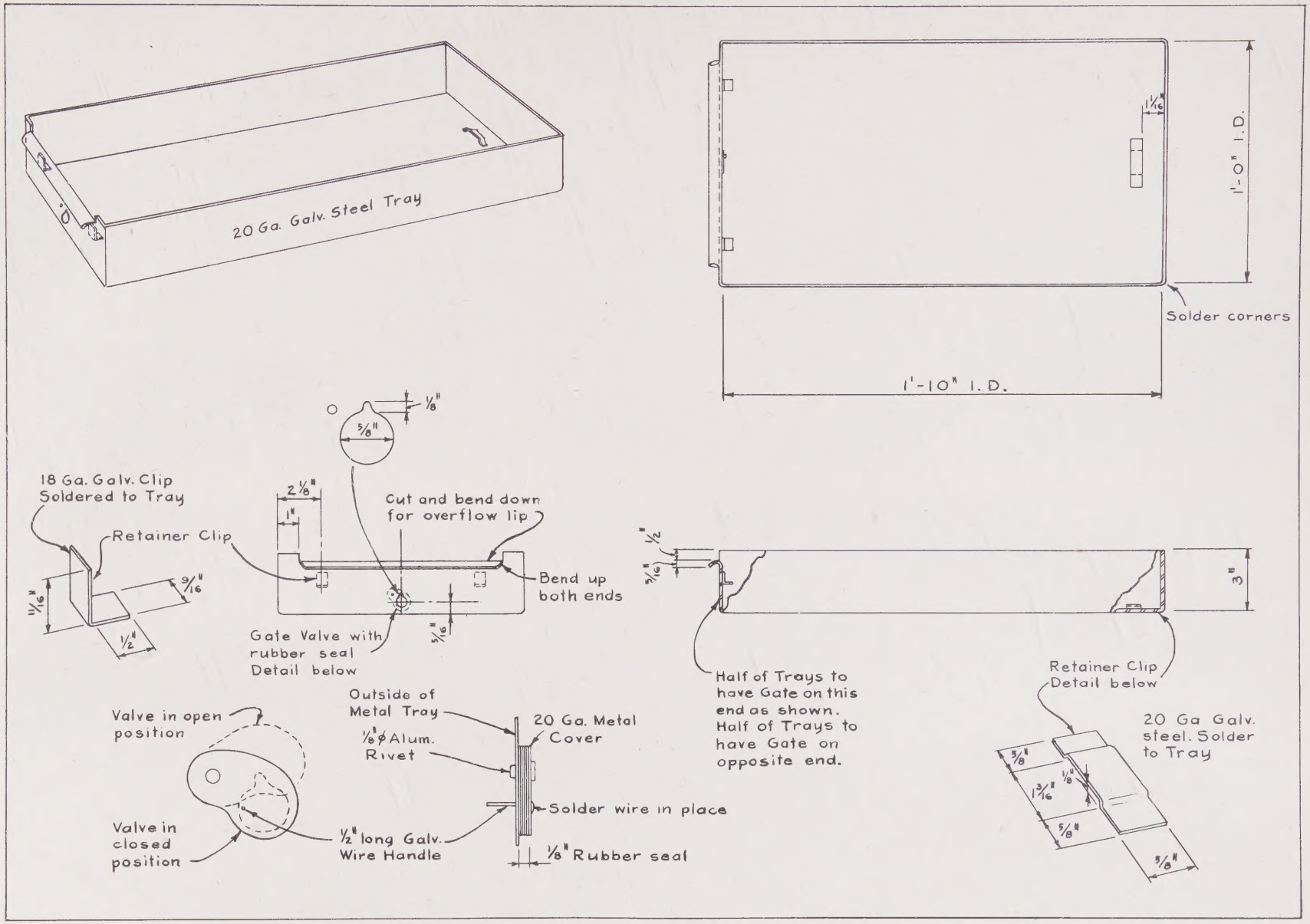
20 Ga. Galv. Steel Tray
1'-0" I.D.
Solder corners
1'-10" I.D.
18 Ga. Galv. Clip Soldered to Tray
Retainer Clip
Cut and bend down for overflow lip
Bend up both ends
Gate Valve with rubber seal Detail below
Half of Trays to have Gate on this end as shown. Half of Trays to have Gate on opposite end.
Retainer Clip Detail below
20 Ga Galv. steel. Solder to Tray
Valve in open position
Valve in closed position
Outside of Metal Tray
20 Ga. Metal Cover
Solder wire in place
Rubber seal

FIGURE 4.--The incubator tray.

basket, the basket cleats have a 1/4-inch upward slope toward the intake end of the basket. Care must be taken to stretch the plastic bottom screen uniformly so that there will be no sag in which air bubbles may collect. The uniform stretch and slope of the bottom screen effectively prevent the formation of air pockets beneath the basket.

The horizontal outlet screen is designed to increase the screen area. To operate effectively, it must be slightly below the normal water surface as controlled by the tray lip. When the outlet screen is installed, care must be taken to assure that the height of the vertical surface does not exceed the specifications shown in figure 3.

The gate valve on the tray--a valve which is placed on opposite ends of consecutive trays so that all the valves are on one end of a battery--is installed so that the water level may be lowered prior to the removal of the tray for cleaning. Though the type of valve shown in figure 4 is effective, there are several other types which might cheapen the cost of construction. A short metal collar could be soldered into the hole in the sheet metal, retaining the approximate size of the hole, and a rubber stopper substituted for the gate. The collar would allow for the easy removal of the stopper and prevent excessive abrasion. Another alternative would be the use of a close nipple and a pipe cap. There is no objection to the free flow of water through the gate, as the plastic bottom screen is of mesh fine enough so that there is no danger of injuring the fry sacs; therefore, the control of water attained by the sliding gate is not necessary.

Most of the details of construction are adequately shown in figures 1 through 4, and further explanation would be redundant. However, one dimension was inadvertently omitted and one specification was purposely omitted. The space between staggered tray stops on the incubator frame is 1 inch. This 1-inch space permits the water spilling from the tray above to be collected at the intake end of the tray underneath. The specification as to the size of the main intake line and individual feeder lines was omitted purposely. The size of these lines will depend on the head that is available. With 4 feet of head, a 2-inch main line and 1/2-inch feeder lines were adequate.

It is recommended that the basket be painted only on the inside with two coats of paint. The common practice, in hatcheries, of painting all sides of submerged wood should be avoided if dry rot is to be prevented. The tray, which is made of metal, should be painted with two coats on all sides to prevent rust and shield the galvanizing.

One of the important advantages of this type of vertical incubator is that it is easily constructed. A complete 10-bank incubator was made at the Salmon-Cultural Laboratory with no outside assistance. The baskets, being made principally of wood, require only a good table saw and a minimum of carpenter's tools plus accurate workmanship. Unless a metal break is available, the trays should be formed in a sheet-metal shop to assure sharp right angles at the bends. The frame may be either welded or riveted, but it must be level and the slides true. If welded construction is used, care must be taken to assure that the frame and slides are not warped by excessive heat.

Operation

The vertical incubator requires but a minimum of attention during the greater part of the incubation period. There are, however, certain procedures for which techniques have been developed to facilitate operations and certain alterations which can be made in the incubator to meet specific conditions.

The incubator, as shown in figure 1, is designed to operate with 15 baskets containing eggs. The top tray is carried as a blank to allow for the dissipation of air bubbles appearing in the feeder line. The capacity of the baskets is 80 displacement ounces, 80 ounces by weight, or 120 egg-level ounces. These various methods of measurement have been described by Burrows (1951). Before the incubator is stocked, the baskets are placed in the

picking trough and the measured eggs are placed in them. The well at the lower end of the trough is used to receive the eggs and to retain them during measurement. The dimensions of the well vary with the size of the egg-taking operation.

The loaded baskets are locked into the empty trays and then placed in the incubator. When the take of eggs is not large enough to fill one bank completely, it is advisable to load from the bottom up. With this procedure, the cleaning of the screens during hatching is reduced to a minimum. When a bank of trays is not completed in one day, a blank tray is placed above the last full basket and a hose attached to the intake pipe to deliver the water to the blank tray. In this manner the free fall and excessive splashing of the intake supply are eliminated. A 5-foot length of 3/4-inch soft rubber tubing is adequate for this purpose when 1/2-inch delivery pipes are used.

Fungus-control treatments using malachite green as described by Burrows (1949) may be administered by placing the siphon bottle so that the delivery is directly into the top blank tray. It is recommended that the water inflow be continued for the full hour-long treatment period to avoid the danger of oxygen depletion. Even with this procedure, the amount of malachite green required for treatment is reduced by half because the water inflow is but a quarter of the conventional requirement.

From the time the eggs are taken until the fry are removed from the baskets for feeding, but two pickoffs are necessary under normal conditions. The first pick-off is made when the eggs are shocked just prior to hatching. When the eggs are removed from the basket, it should be thoroughly cleaned to remove any sediment or slime mold which may have accumulated. After hatching is complete, the baskets should again be placed in the picking trough, the mortality removed, and the basket screens cleaned. The trays also should be cleaned of excess shells at this time. High mortalities or cold water temperatures may make it desirable to clean the baskets an additional time during the egg stage, usually just subsequent to the critical period. If the incubation period is prolonged because of water temperature of 45° F. or below, slime molds along the sides of the baskets may develop to such an extent as to obstruct the flow of water through the eggs. Under these circumstances, the baskets should be cleaned as soon as egg development has progressed to the closing of the blastopore or the end of the critical period.

The gate valve at the end of each tray is installed to facilitate removal of the trays from the incubator. When it is desired to remove a bank of trays, the intake water is shut off and the tray valves are opened, starting with the bottom tray and progressing to the top. The lowering of the water makes the trays lighter and reduces the danger of spilling eggs or fry as the trays are handled. After the water level is reduced, the trays are taken from the incubator and the baskets are removed and placed in the picking trough, where they may remain for an indefinite period.

The picking trough operates with running water introduced at a rate comparable to that of the incubator. The dam board in each section is of a height sufficient to maintain the water level over the horizontal outlet screen. Wedges are used to secure the baskets in position against the dam boards and tightly against the bottom so that the water is directed up through the bottom screen and complete circulation is assured.

The outlet screens of the baskets require little or no attention until the eggs start to hatch. From this time until the hatching is completed, the screens must be cleaned 3 to 4 times a day, dependent on the rapidity of hatch. To clean the screens, a test-tube or bottle brush 1-1/2 inches in diameter has been very satisfactory. This type of brush has a twisted wire handle which may be stiffened with a 3-1/2-inch length of copper tubing (outside diameter, 1/4 inch). The brush and tube are mounted on a 5-foot light wooden handle, at right angles to the handle. The copper tube is inserted through a hole at one end of the handle; then the brush handle is brought through the tube, bent, and secured to the wooden handle. The 5-foot

handle allows for the cleaning of the basket screens which are on the inside of the battery. The brush is inserted into the outlet end of the tray, and the horizontal basket screen is cleaned with a vigorous back-and-forth motion of the brush.

The hatching period may present a critical cleaning problem under certain conditions. Egg-takes large enough to fill one bank of baskets may require additional cleaning of screens to remove the egg shells. Under these circumstances, it may be necessary to clean the outlet screens at 3-hour intervals during the day and late in the evening and early in the morning to prevent the screens from plugging. Where large egg-takes are anticipated, there is a second means of circumventing this difficulty. The outlet screen may be altered to increase the screen surface by extending the horizontal surface to cover the basket completely. In this alteration two screens are used. The vertical screen is permanent and of the same dimensions as those shown for the end screen in figure 3. The horizontal screen is removable, sliding in saw cuts 1/8 inch deep in the sides and end of the basket parallel to, and 3/4 inch below, the top of the basket. The horizontal screen is 10-1/2 inches wide and 19-1/2 inches long. To stiffen the end which overlaps the vertical screen and provide a handhold for the removal of the horizontal screen, a 1/2-inch right-angled downward bend is made at the outflow end of the screen. Notches 1/8 inch by 1/2 inch are cut from this end of the screen to allow for the bend. The additional 1/2-inch bend brings to 20 inches the over-all length of the material required for the horizontal screen. Both vertical and horizontal screens are made from perforated aluminum plate of the same specifications as shown in figure 3.

There are certain advantages and disadvantages to the use of a complete horizontal outflow screen. First and foremost of the advantages is that the screens require little or no cleaning at any time during the incubation period. Also, the fry are completely confined, so that baskets may be handled with less care than when the fry are exposed. A third advantage is that with the horizontal screen removed, all portions of the basket are exposed for the removal of mortalities. The principal disadvantage is that the eggs and fry are not open to inspection without removal of the basket from the tray, which, of course, involves removal from the incubator. A second disadvantage is the 75-percent increase in the amount of perforated screen required. It is apparent, however, that in large-scale operations, the reduction in the care required for operation far outweighs the disadvantages mentioned.

The incubator will handle with no difficulty the slight amount of silt occurring in a spring water supply. If, however, a substantial amount of silt and debris is to be expected, the upper tray should be converted into a filter. A basket frame is altered by substituting a dam board of frame lumber for the outlet screen and removing the end cleat from the bottom of the basket. The dam board extends flush with the top of the basket frame. The plastic screen is placed on the bottom of the basket, and 3 additional 1/2-inch frames covered with plastic screen are inserted above the bottom screen. A 1/2-inch cleat above the bottom screen holds the adjacent frame so that 4 horizontal screens, 1/2 inch apart, occur in series within the basket frame. These frames are held in place by pins inserted into holes in the center of each end of the top screen frame and extending into matching holes in the basket frame. The normal water flow through a basket is reversed in the filter, the water being introduced in the center of the top screen frame, passing through the 4 screens, and out underneath the basket. The screen frames of the filter may be removed and cleaned as often as conditions warrant. When the water is extremely turbid and debris-laden, the small amount of water required for the incubator makes it entirely practicable to install a more complex filtering system in the incubator supply line.

A 10-bank incubator has been in production operation at the Salmon-Cultural Laboratory for the past 2 years. Approximately 1,500,000 chinook salmon eggs and

fry and 600,000 blueback salmon eggs and fry have been incubated by this method. The incubator has proved entirely practicable in production operation. The man-hours required for its operation compare very favorably with any of the conventional methods of egg and fry incubation.

Acknowledgment

The assistance of Harold G. Kronholm and Trevor F. Watson in the construction and design of the incubator is gratefully acknowledged.

Summary

A vertical incubator for salmon eggs and fry has been designed, constructed, and tested under hatchery production conditions. The incubator has proved entirely practicable and may be adapted to meet specific requirements. The most efficient of conventional equipment requires four times the floor space and at least four times the water supply of the vertical incubator. Undoubtedly, this equipment could also be used for trout, particularly those species that have large eggs.

Literature Cited

Burrows, R. E.
1949. Prophylactic treatment for control of fungus (Saprolegnia parasitica) on salmon eggs. Prog. Fish-Cult. 11 (2): 97-103.

1951. An evaluation of methods of egg enumeration. Prog. Fish-Cult. 13 (2): 79-85.

Nordqvist, Oscar.
1893. Some notes about American fish-culture. Bull. U. S. Fish Comm. 13: 197-200.

Schaeperclaus, Wilhelm.
1933. Lehrbuch der teichwistshaft. (Textbook of pond culture). Berlin, 260 pp., illus. (U. S. D. I., Fish and Wildlife Service, Fish. Leaflet 311).

Simon, J. R., and Floyd Roberts.
1941. A drip incubator and heater combination. Prog. Fish-Cult. 56: 10-13.

ASSISTANCE FOR SHAD RUN ON THE ATLANTIC COAST

Since the building of the Holyoke Dam on the Connecticut River in 1848, very few shad had been able to reach their ancestral spawning grounds between Holyoke and Turners Falls, Massachusetts. Between May 23 and June 28, 1955, however, 4,902 shad were passed over this barrier so that these valuable fish might spawn in the upper waters.

At the Holyoke Dam, shad ascending the river to spawn were attracted to a latticelike gate through which water was flowing. Behind the shad so attracted to the first gate, another gate dropped, trapping the fish in an 8- by 8-foot area. The hopper at the bottom of this elevator shaft was then lifted about 20 feet, or to within a few feet of the surface. From the hopper, the fish were easily transferred with nets to large buckets. Next, these buckets were moved--with as little delay as possible because the shad quickly used up the oxygen in the small amount of water in the buckets--along a ramp from the side of the hopper to a spot some 50 feet higher than the hopper and behind the dam. Here the shad were returned to the river.

This fish-passage arrangement at the Holyoke Dam was probably the only one operating on the Atlantic coast to move large numbers of shad upstream past a dam.

TRACING THE COURSE OF HATCHERY GROUND WATER SUPPLIES WITH AN IMPROVED FLUORESCEIN CONCENTRATION METHOD

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and
Thomas Iezzi, Rohm and Haas Company, Bristol, Pennsylvania

BLUE SPRING, ONE OF THE MAJOR SOURCES OF WATER SUPPLY for the Pennsylvania State Fish Hatchery situated at Bellefonte, Pennsylvania, unexpectedly began discharging extremely muddy water. The location of the source of this damaging pollution instituted an intensive investigation of the watershed for the spring. The dye fluorescein, or uranin, was used extensively. Much experience was gained in its use, and an improvement was made in the detection of the dye. The authors feel that this paper will be of value to fishery men, not only because of the practical uses found for fluorescein, but also because of the scarcity of literature on the subject.

The use of common salt, as reported by Tolman (1937) was one of the early methods of measuring the movement of ground water. Salt was introduced in sinkholes or test wells, and attempts were made to detect the chemical in other test wells. The velocity and direction of flow could thus be established, provided that the salt could be detected. Slichter (1905) employed a method utilizing a strong electrolyte and electrical connection between test wells. The movement of water containing the chemical could be detected with an ammeter. Other materials used in tracing passage of underground water were insoluble starches, cultures of bacteria, and dyes.

The coal-tar fluorescein was found to be among the best of the dyes because of its brilliance, extreme solubility in water, and capability of traveling long distances without being destroyed by comparatively long contact with underground water and environs. Dole (1906) reported its use in Europe for tracing the passages of pollution from sewers as early as 1887. Since then the use of the dye for tracing ground water and pollution has been well established. However, actual directions for use of the dye are hard to find. A number of texts and reference books on ground water, hydrology, and sanitation were perused for such information without success. Fluorescein was merely mentioned in most of these books.

The Bellefonte Hatchery is in a limestone region characterized by dry hollows, underground streams, sinkholes, and large springs. These four characteristics are related. The dry hollows are often drained by underground streams directly or through sinkholes. The underground

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This paper was presented at the Conference of the Northeast Section of the Wildlife Society; American Fisheries Society; Fish, Game, and Conservation Commissioners; and the Game Breeders' Association of New Jersey--March 23-25, 1955, Atlantic City.

The junior author was formerly sanitary engineer with the Pennsylvania Department of Health.

streams are carried in channels worn by solution and erosion through limestone-bearing layers. The sinkholes are surface cave-ins resulting from structural failures caused by continuous channeling. The sinkholes, therefore, often mark the presence of an active underground stream. The point of emergence of a stream to the ground surface is, of course, a spring.

The underground streams may travel for long distances and have an extensive, intricate tributary system draining many square miles. The springs supplied by these streams are often large. Within a 5-mile radius of the Bellefonte Hatchery there are no less than 8 springs having capacities ranging from 1,000 to 11,000 gallons per minute.

The limestone springs are desirable for trout-hatchery use because of their large sustained flows and rather constant, near-optimum temperatures throughout the year. However, an undesirable feature is the ease with which the springs may be polluted. In general, if the underground streams are relatively close to the ground surface, the number of sinkholes can be great. In the watershed area for Blue Spring, much of the surface water flows but a short distance before sinking beneath the surface without the benefit of natural filtration.

The determination and investigation of a watershed for a spring can be time-consuming. The dye may be added at only one point on the suspected watershed at a time. Therefore, in order to avoid unnecessary work, considerable time and thought should be given to preparing a plan of approach. A topographical map should be studied and the area investigated. Significant points such as sinkholes, draws, ground depressions, and springs should be plotted. A knowledge of the geological structure of the area is very helpful. It might be advisable to consult a geologist in formulating a plan.

After a probable key point is selected, the dye is added, samples are collected at strategic and possible discharge points, and analyses are made. Of course, no analysis need be made of a sample when the dye is visible.

The amount of dye to use is not very critical but this can be ascertained to some extent by local conditions. In the Bellefonte area, experience indicates that 1 to 4 pounds of dye are sufficient for most tracing work. A small amount of fluorescein goes a long way. The least amount that can be readily detected with the naked eye is about 1 part in 5 million. A trained observer using Nessler tubes might detect the dye in a concentration as low as 1 part in 40 million. The presence of dye can be detected in much smaller amounts by concentration methods.

The Lombard method of concentration is given by DeMent (1945). In that method ether and ammonium hydroxide are used to extract the dye. This procedure was reported to permit detection of dye in a concentration of 1 part in 200 million.

The method employed at the Bellefonte Hatchery is a procedure used by the Pennsylvania Department of Health. This method makes possible detection of as little as 1 part in 2 billion. By supplementing this method with a carbon arc-lamp, the sensitivity can be increased to pick up a dye concentration of 1 part in 20 billion. An experienced observer can detect fluorescein in even much smaller amounts. Furthermore, the method is sufficiently flexible so that greater sensitivity can be procured by increasing the sample volumes, number of extractions, and so forth.

Briefly, the test consists of adding acid to a water sample to render the dye less soluble, extracting with an organic solvent that is not soluble in water, separating the solvent layer from the water into a test tube, and rendering it alkaline with sodium hydroxide. The small amount of water that is added in rendering the solvent layer alkaline assumes the characteristic green color as it takes up the dye.

Gallon jugs or 3-quart reagent bottles make satisfactory sampling containers. Amyl acetate, which is sometimes called pear oil or banana oil, is a very satisfactory organic solvent and is readily obtained from chemical-supply houses, paint-supply houses, and drug stores.

During the experiments it was found that a carbon arc-lamp with a focusing device, such as is used for microscope illumination, increases the sensitivity of the concentration method by more than 10 fold. The intense illumination of the carbon arc focused on the water layer in the test tube will reveal the green color of the dye at levels not detected with the naked eye or other types of illumination. The test tube can be viewed against a background of white or black, according to individual preference. Details of this test are appended to this paper.

Muddy water is, of course, highly undesirable in a trout hatchery. It is particularly harmful to eggs and young fish. When the Blue Spring unexpectedly began to discharge water having the appearance of a mud slurry, there was much concern. An intensive search of the area was made for some miles around to try to pin-point the source of pollution.

It was learned that the mining operations of a new lime plant, located about 3 miles from the spring, had encountered such amounts of ground water that continuous pumping with large-volume apparatus was necessary. The plant pumped the water from the mine, through a short distance of pipe, into a sinkhole. This was sufficient information to prompt a thorough investigation of the pumping activities at the lime plant. Aid from the Pennsylvania Department of Health and from the State Bureau of Topographic and Geologic Survey was obtained.

Personnel of the Department of Health, the Fish Commission, and the Bureau of Topographic and Geologic Survey set up a program to determine whether the mine water was entering the feeder streams of Blue Spring. A quick geological survey was made, and sampling points were selected. Two pounds of dye were added to the sinkhole at the mine. A series of water samples was collected from Blue Spring, a well, and a number of other springs in the area. Sampling intervals ran from 1 hour for the Blue Spring to about 3 hours for other points. These samples were analyzed at the fishery laboratory and at the Sanitary Engineering Laboratory of the Pennsylvania State University.

The well between Blue Spring and the mine gave a positive test in a little over 2 hours. Dye was picked up in the Blue Spring sample after 13 hours.

Since it had been shown that the mine pumpings were entering Blue Spring, the lime-plant officials were questioned about past operations. It was revealed that a large mud-filled seam was exposed during their mining operations. The mud was not removed by mechanical means but was "puddled" and "mucked" or pumped out into the sinkhole. The mystery of the extremely muddy water was thus solved.

The amyl acetate concentration method supplemented with a carbon arc-lamp did prove valuable. However, the great sensitivity of the method requires caution. Experience in running the dye test was, as in most work, the greatest teacher. It was learned the hard way that the person adding the dye should be someone not connected with any part of the collecting and analyzing of samples.

Wally added the dye. Wally stood downwind when he added the dye. Wally was contaminated from head to foot with fluorescein dust. Wally walked around the laboratory where the samples were being tested. The result was a contaminated laboratory. Wally's dust was found in the analysis. The reagents were contaminated: everything -- even the distilled water -- gave a positive test. The entire testing program was moved to the Sanitary Engineering Laboratory at the Pennsylvania State University, about 10 miles distant. Here clean, non-contaminated reagents and glassware were available to complete the tests. In subsequent tests the dye was mixed with water and added to the sinkholes as a slurry, not in a dry state. Of course, Wally did not collect samples or enter the laboratory.

After the initial test, the lime-plant was interested in learning whether part of the pumped water was being recirculated and also the source of water running into the mine. The plant made additional dye tests in surrounding sinkholes. It was found that water reached the mine from

several sinks as much as 2 miles away. The time of appearance of dye in the mine ranged from 9 hours for the closest sinkhole to 31 hours for the farthest tested.

The Fish Commission was also interested at this point in knowing more about the watershed of the spring and conducted other dye tests in cooperation with the lime-plant officials. Two other areas, in directions other than the lime plant, were established as contributors to Blue Spring. A sink in each of these areas carried away the water from two mountain streams.

The experience gained in the use of fluorescein proved of value on three other occasions. On the first, the Fish Commission had let a contract to repair a dam at one of its lakes. The contractor was to repair the spillway and install a pipe at the bottom of the dam so that the lake could be drawn down. The pipe led to a narrow passage which was enclosed with stop planks. After the work was completed, a leak developed through the walls of the dam near the drainpipe. The contractor claimed that the leak had been caused by defects and seepage in the old existing dam structure and would assume no responsibility in the matter. Fish Commission personnel, however, were quite sure that the leaks were caused by faulty installation of joints in the concrete drainpipe. A dye test was arranged in the presence of a State inspector and representatives of the contractor. Fluorescein was introduced into the drainpipe with the aid of a smaller pipe. A short time later the leaks showed the characteristic green color and the contractor's negligence was established.

On another occasion a dye test helped to prove that a flow of water below a dam was not a leak but merely a spring.

In February 1955, one of the Commission's recently developed limestone springs flowed water of high turbidity after rains or heavy thaws. A sinkhole in a plowed field was discovered about 1 mile away. Two pounds of fluorescein was added to this hole immediately after a heavy rain. The dye was visible in the spring in 24 hours. It is hoped that some arrangement can be made to change the farming practice around the sink area in order to alleviate the soil contamination of this spring.

The employment of the dye fluorescein in tracing water movement proved to be a useful tool for the Pennsylvania Fish Commission. The examples presented show how some of the Commission's most valuable assets were protected through its use. The knowledge of ground water movement in a watershed area was broadened considerably through dye detection with the concentration method, supplemented with a focused carbon arc-light source. The increased sensitivity of detection resulted in greater analytical efficiency and more reliable data.

Literature Cited

Tolman, C. F.
1937. Ground water. 593 pp., illus. McGraw Hill Book Co., N. Y.

Slichter, C. S.
1905. Field measurement of rate of movement of underground waters. U. S. Geol. Surv. Water Supply Paper 140: 16-28.

Dole, R. B.
1906. Use of fluorescein in the study of underground water. U. S. Geol. Surv. Water Supply Paper 160: 73-85.

DeMent, J. A.
1945. Fluorochemistry. 796 pp., illus. Chemical Publishing Co., Inc., Brooklyn, N. Y.

APPENDIX

Use and Detection of the Dye Fluorescein in Tracing Water Movement[1/]

Use of the dye fluorescein, or uranin, in tracing the passage of pollution from surface water, sewers, cesspools, tile

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1/ This procedure, with modifications, is used by the Pennsylvania Department of Health.

drains, and so forth into ground waters tributary to springs, wells, or surface waters has been well established. This dye imparts to water a fluorescent green color which is apparent to the eye when the dye is present even in small concentrations. The color becomes much less distinct, however, with greater dilutions; hence, without its being apparent to the eye, dye may be present in samples of water collected for examination. The following procedure has been developed to increase the sensitivity and effectiveness of this test by concentrating the dye in a small volume of an organic solvent.

Though this is primarily a laboratory procedure, it can be utilized as a field test. The test method will develop an observable color when the dye is present in the water in amounts as low as 0.0005 part per million, which would not be visible in the untreated sample.

The technical background for the test may be explained in one of two ways:

(1) Uranin is the sodium salt of fluorescein. "The fluorescent properties of all alkaline solutions of fluorescein and related dyes result from an interchange of H+ and OH- within the molecule wherein the H+ is shifted from the phenolic hydroxyl to the carboxyl oxygen of the quinoid system with a resulting shift of electrons along the 11-atom system terminating by 2 oxygen atoms."

(2) An alternative explanation is: "The quinoid form in which fluorescein exists as the disodium salt in an orange red powder which dissolves readily in water to form fluorescent solutions. Upon treatment with acids the quinoid tautomer is destroyed and the fluorescence disappears."

In the form of the sodium salt, fluorescein is very soluble in water; but when the salt is acidified, the water solubility greatly decreases, although it remains quite soluble in certain organic solvents such as ether and amyl acetate (sometimes called pear oil or banana oil).

The test consists of adding acid to the water to render the dye less soluble, extracting with the organic solvent which is immiscible with water, separating the solvent layer from the water, and rendering it alkaline with sodium hydroxide. The small amount of water added in rendering the solvent layer alkaline assumes the familiar fluorescein green of the sodium salt of the dye.

Preliminary Test

Sometimes fluorescein is not visible in water simply because the pH is too low, in which case the addition of sufficient alkali to the water will make the fluorescein easily visible. Therefore, sufficient sodium hydroxide solution is added to the sample in a clear, uncolored 1-gallon bottle (have the bottle about three-quarters full) to cause a drop of the well-mixed water to leave a blue spot on a piece of red litmus paper. One or two milliliters of a normal solution of sodium hydroxide will usually be sufficient.

Place the bottle in a ray of sunlight or, if that is not available, hold a strong light--a flashlight will do--close against the bottle and look through it toward a dark or white background, according to preference. The line of vision should be at right angles to the path of the light beam. Use of a focused carbon arc-lamp (such as used for microscope illumination) for a light source greatly facilitates detection, particularly at lower concentrations. Therefore, it is possible that the numbers of samples to be analyzed by concentration can be reduced.

Further Procedure

If the dye is not readily visible with the preliminary test, the same sample may be treated by the extraction procedure, as follows:

Add sufficient acid to the sample in the bottle to cause a drop of the well-mixed water to leave a pink spot on a piece of blue litmus paper. One or two milliliters of a normal sulfuric acid solution will usually be sufficient, but a little more may be required if an excess of alkali has been added in the preliminary test.

Add about 30 milliliters (or 1 ounce) of amyl acetate to the bottle and shake thoroughly for one-half minute. Add more water to bring the amyl acetate layer--which floats on top of the water--up into the narrow part of the bottle where the stopper goes. Allow to stand for 10 to 15 minutes to permit separation of the 2 phases.

Carefully remove the amyl acetate layer with a medicine dropper or pipette and transfer it to a clear, untinted test tube. A slight amount of water carried over will do no harm, but an effort should be made to secure all that is possible of the solvent.

Add to the removed solvent sufficient sodium hydroxide (1 milliliter of a normal solution is usually sufficient) to cause a drop of the water layer on the bottom to leave a blue spot on red litmus paper. Mix the water layer and the solvent thoroughly by shaking. Allow to stand about 15 minutes; then view as directed in the preliminary test. Fluorescein will be readily visible by normal methods unless its concentration in the original sample is less than 0.0005 part per million. However, a concentration of 0.00005 part per million is easily detected with a focused carbon arc-lamp as a light source.

The sensitivity of the test may be increased further by using larger sample volumes, more extractions, separatory funnels, and so forth.

A commercial form of amyl acetate, known as pear oil or banana oil and used as a thinner for certain paints or lacquers, may be used in the test.

Precautions

1. In order to prevent contamination, the person adding the dye at a point of application should not collect or analyze samples.

2. Only thoroughly clean glassware should be used. The glassware used for the tests yielding negative results may be used over again without much concern over cleanliness.

3. The characteristic green seems to diminish on standing. This is particularly true in weak samples. Color test should be run within 24 hours to prevent photodecomposition.

DURATION OF TOXICITY OF MALATHION

Experimental work with malathion has been continued at the Little White Salmon Fish-Cultural Station of the U. S. Fish and Wildlife Service near Cook, Washington. H. E. Johnson, whose work on the toxicity of Malathion 500 to fall chinook salmon fingerlings has been reported in The Progressive Fish-Culturist (1955: 113), has recently disclosed the results of tests undertaken to determine whether malathion could be substituted for rotenone in eliminating undesirable populations.

The drains in a number of standard hatchery troughs were plugged and the water inflow was shut off when the water reached a depth of 30 centimeters. A wettable powder that contained 25 percent malathion, made up for use as an insecticide, was dissolved in the test troughs to make concentrations of 0.32, 0.24, and 0.18 part per million. On that same day--and on the 1st, 2d, 3d, 7th, and 14th days thereafter--20 fall chinook salmon fingerlings were put in each trough that received malathion and in a control trough. The number of dead in each trough was noted 6, 24, and 48 hours after each introduction.

The duration of toxicity was such that 19 of the 20 fingerlings were dead within 24 hours after being put in the trough that had received a concentration of 0.32 part per million 14 days earlier. Similarly, the concentration of 0.18 part per million, which was fatal to 14 fingerlings in a 48-hour exposure in the first test, was fatal to the same number in the last.

T-O_2, AN INSTRUMENT FOR THE ESTIMATION OF TEMPERATURE AND DISSOLVED OXYGEN IN NATURAL WATERS

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VARIATIONS IN THERMAL STRATIFICATION AND DISSOLVED OXYGEN CONTENT IN VERTICAL SERIES OF SAMPLES are of prime importance to biologists in their efforts to characterize standing waters. Differences in temperature and dissolved oxygen determine, at least in part, the distribution of all aquatic organisms. Temperature and oxygen curves have been utilized by Hutchinson (1938) in comparisons of the relative productivity of lakes by means of the areal hypolimnetic oxygen deficit. An instrument which combines estimates of temperature and relative values of dissolved oxygen has been used by the Fishery Survey Unit of the Rhode Island Division of Fish and Game as a means of expediting the mechanics of gathering these data.

Ohle (1953) has adequately described the theory and operation of an instrument for the electrochemical determination of the dissolved oxygen in natural water. Mortimer and Moore (1953) have described the theory and construction of thermistor thermometers for temperature measurements in water. The instrument described herein combines a simple thermistor circuit with an electrolytic cell, similar in principle to that described by Ohle, in a single unit as shown in figure 1. This instrument has received a limited amount of both field and laboratory usage, giving satisfactory results for the purposes at hand, namely, the field estimation of the vertical distribution of temperature and dissolved oxygen.

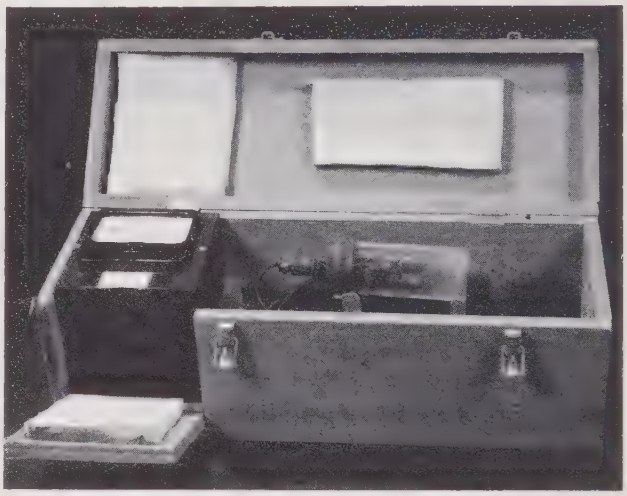

FIGURE 1.--The instrument used in the recent investigation. The unit is approximately 8 by 8 by 24 inches and weighs 6 pounds.

Description of Instrument

Figure 2 illustrates by schematic diagrams the circuits used for the thermistor

Federal Aid to Fish Restoration funds were used for this problem under Dingell-Johnson Project No. F-2-R-3.

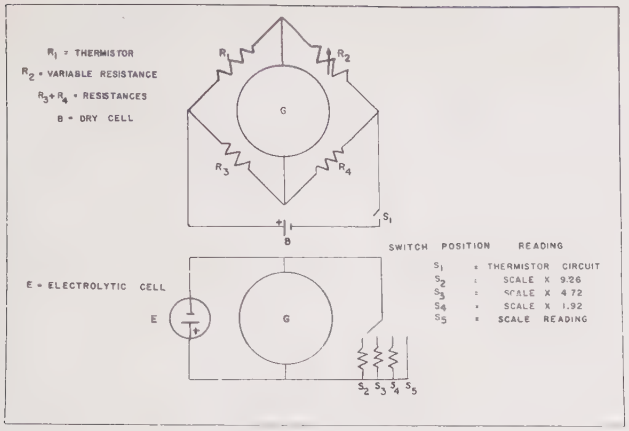

FIGURE 2.--Thermistor and electrolytic cell circuits of instrument illustrated. The same microammeter is used for both circuits.

(Illustrations courtesy of the author)

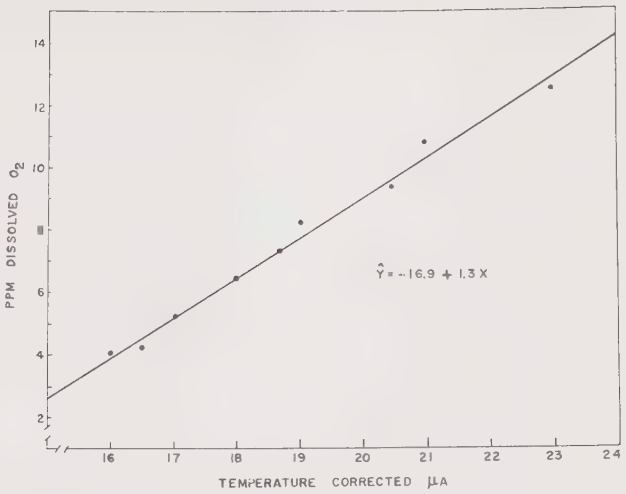

FIGURE 3.--The relationship between dissolved oxygen and temperature-corrected microampere readings under laboratory conditions.

thermometer and the electrolytic cell. The following describes the electrolytic cell of this instrument in further detail:

Cathode --------	10 K gold alloy, 1.2 x 1.5 x 0.15 cm.
Anode ----------	Zinc, 2.0 x 2.5 x 0.1 cm.
Spacing between electrodes. --------	1.2 cm.
Electrode housing. ------	Polystyrene cylinder, 5 cm. diameter x 15 cm. high
Cable ----------	50 feet of insulated 4-core, No. 18 wire (Belden 8454)
Microammeter ---	Simpson No. 29. Full-scale deflection equal to 25 units.

The circuit of the electrolytic cell is equipped with shunts to vary sensitivity over a range of about nine times the scale reading. A rotary switch permits changes from positions 2 through 5, shown in figure 2; so that nearly full-scale deflection can be obtained over a variety of conditions with respect to the dissolved oxygen content and temperature of the water.

Position 1 of the rotary switch closes the thermistor circuit. The thermistor element is located slightly above the electrodes in the polystyrene cylinder, and both circuits are connected by 50 feet of 4-way cable to the microammeter. In this instrument, full scale of the microammeter was adjusted to a temperature of 35° Centigrade by means of the variable resistance of the bridge circuit. A calibration curve was then made to relate microammeter readings to observed temperatures. The response time of this thermistor was about 30 seconds, and an accuracy of approximately $\pm 1/4^{\circ}$ was attained. Obviously, the accuracy and response time of the thermistor may be altered within reasonable limits according to the size and kind of elements used.

Use of the Instrument

In field operation, the temperature was first measured by turning the rotary switch to S_1 (thermistor) position and recording the scale reading for a vertical series of measurements, usually at one-half-meter intervals. The rotary switch was then turned until the electrolytic-cell reading was nearly full-scale deflection at the surface while the immersion assembly

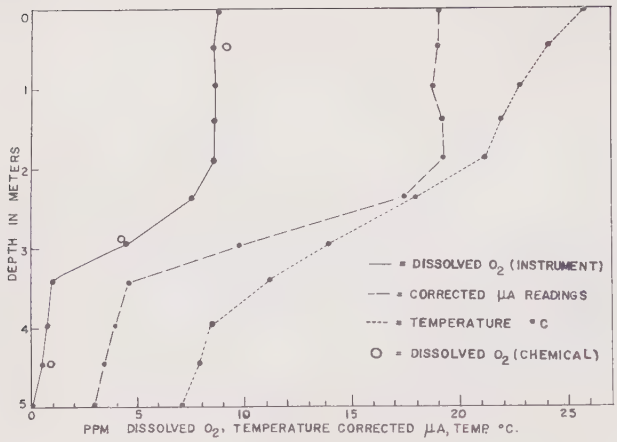
DEPTH IN METERS
PPM DISSOLVED O_2, TEMPERATURE CORRECTED μA, TEMP. °C.
— = DISSOLVED O_2 (INSTRUMENT)
— = CORRECTED μA READINGS
---- = TEMPERATURE °C
O = DISSOLVED O_2 (CHEMICAL)

FIGURE 4.--Deep Pond, Arcadia, Rhode Island, August 24, 1954. The relationships among temperature, temperature-corrected instrument readings, and oxygen values.

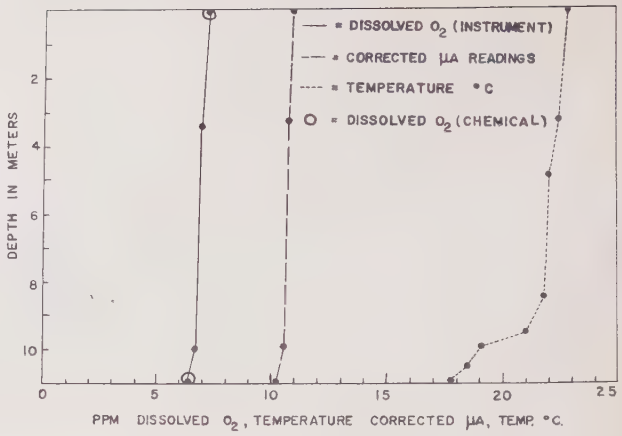
DEPTH IN METERS
PPM DISSOLVED O_2, TEMPERATURE CORRECTED μA, TEMP. °C.
— = DISSOLVED O_2 (INSTRUMENT)
— = CORRECTED μA READINGS
---- = TEMPERATURE °C
O = DISSOLVED O_2 (CHEMICAL)

FIGURE 5.--Watchaug Pond, Charlestown, Rhode Island, August 27, 1954. The relationships among temperature, temperature-corrected instrument readings, and oxygen values.

was being moved gently up and down. Readings were then obtained at intervals until a vertical series of measurements had been made on the electrolytic-cell circuit.

The combined method described by Ohle was used exclusively for relating the temperature-corrected microammeter readings of the electrolytic-cell circuit to dissolved oxygen during field use. This consisted of analyzing two or three water samples for dissolved oxygen by conventional chemical methods and using these results to calibrate the temperature-corrected current readings. For a rough-and-ready classification of standing waters into trout and nontrout habitat, no calibration seems required--as the following figures will illustrate.

Experimental Work

Some indication of the accuracy of the instrument is given. Figure 3 illustrates the relationship between instrument readings converted to 15° C. and values of dissolved oxygen expressed as parts per million. The water in this experiment was tap water (R_{18} = 3,000, Total alkalinity = 0.9 p.p.m.), and oxygen determinations were made by the unmodified Winkler method. The relationship between the temperature-corrected instrument reading and dissolved oxygen appeared linear, and a regression line was fitted through the scatter of points obtained. The standard deviation from regression was 0.3 p.p.m. in this instance.

Examples of field data are given in figures 4 and 5. In figure 4, it is evident that the three curves illustrated are somewhat similar in shape, and the values of dissolved oxygen in the lowest stratum estimated by both the chemical and the instrumental method appear very low. Evidence of both chemical and thermal stratification is suggested here. The temperature curve of figure 5 also gives some evidence of thermal stratification. However, the instrument-determined oxygen curve and the chemically determined values are similar but do not approximate the temperature curve. No severe oxygen depletion has been indicated in this example. The results that have been obtained here in two figures suggest that temperature-corrected instrument readings alone may be used to characterize a body of standing water with respect to the vertical distribution of dissolved oxygen.

Discussion

Initial efforts at construction and use of an instrument combining temperature and oxygen measurement have met with a reasonable degree of success. It would seem, from the foregoing experience, that fishery workers can construct, at a moderate cost, similar equipment which may assist in the progress of fishery research and management.

Acknowledgment

The author gratefully acknowledges the assistance of Dr. Arthur L. Quirk, director, Upper Air Research Laboratory, Rhode Island University, in this project.

Literature Cited

Hutchinson, G. E.
1938. On the relation between the oxygen deficit and the productivity and typology of lakes. Intern. Rev. ges. Hydrobiol. u. Hydrogr. 36: 336-355.

Mortimer, C. H., and W. H. Moore.
1953. The use of thermistors for the measurement of lake temperatures. Com. 2, Intern. Assoc. Theor. and Appl. Limn., 25 pp.

Ohle, W.
1953. Die chemische und die elektrochemische Bestimmung des molekular gelösten Sauerstoffesder Binnengewässer. Com. 3, Intern. Assoc. Theor. and Appl. Limn., 44 pp.

DISEASE INHIBITION

It has been demonstrated in actual production operations that under comparable conditions certain types of ponds are more resistant to disease development than are others. In experimental tests, the raceway exhibited a greater inhibitory effect on disease than did the circular pool, and the circular pool a greater inhibitory effect than the Foster-Lucas pond. These tests were conducted at the Leavenworth Station (Wash.), where bacterial gill disease appears to be endemic in the water supply and routine weekly prophylaxis is necessary to prevent the disease from reaching epidemic proportions in Foster-Lucas ponds. Under normal operating conditions without weekly prophylaxis at temperatures approximating 60° F. and with fish loads approximating one-third pound per cubic foot, blueback salmon (Oncorhynchus nerka) contracted gill disease in epidemic proportions in 2 weeks' time. Under comparable conditions, but with stockings of approximately 1 pound of fish per cubic foot, 3 weeks were required for an epidemic to develop in an 18-foot circular pool. In an improvised raceway and in deep troughs with sufficient water inflow to prevent stratification and poundages not in excess of 1 pound of fish per cubic foot, the disease did not reach epidemic proportions and treatments for the control of the disease were not necessary. If water inflows had been reduced in the raceway type proportional to those in the circular or Foster-Lucas ponds--which inflows were comparable--stratification would have developed in the raceway and the disease-inhibition characteristic of this type would have been altered. ...

--R. E. Burrows and H. H. Chenoweth, 1955: "Evaluation of Three Types of Fish Rearing Ponds," Res. Rept. 39, Fish and Wildlife Service, Washington 25, D. C.

HISTOPATHOLOGY OF FISH

I. TECHNIQUES AND PRINCIPLES

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THE TECHNIQUES OF HISTOPATHOLOGY have been used for many years in the study of human and animal diseases. Until very recent times, however, histology has been applied to fish studies only very infrequently. This brief discussion is intended to acquaint the reader with the techniques and principles involved and to explain how histological studies may help to overcome fish diseases and nutritional problems.

The basic building unit of all living matter is the cell. Whether the form of life be fish, human, or plant, its tissues and organs are composed of millions of these tiny units, each living and vibrant. When a fish grows slowly, becomes diseased, or fails to reproduce properly, this is basically due to a misfunction of these tiny cells. When an abnormal fish is cut open for examination, its organs, such as the liver, kidneys, or intestines, may appear damaged. Occasionally such organs are enlarged and fatty, or ulcerated and bloody. Without special techniques, it is difficult to describe accurately even the type of damage, much less the cause of it. Histopathology is one of these special techniques. Histology may be termed the study of cells; pathology, the study of diseases. Histopathology is, therefore, sometimes defined as a study of diseased cells.

In its simplest form, the cell is merely a "glob" of jellylike material, protoplasm. The mass is held together by a cell wall and contains near the center a small, solid particle, the nucleus (figure 1). The nucleus is the controlling mechanism for the cell and is thought by many to be the actual seat of all life processes. In its normal state, the cell might be compared to an active little factory. The raw materials, such as food and oxygen, pass through the cell wall into the protoplasm, where they are converted into energy to move muscles, or into heat, or simply into new cells to produce growth. The nucleus controls these processes. When the cell is healthy and active, it has a very characteristic appearance. The walls are turgid and smooth, the nucleus is clear and distinct, and one can all but visualize the bustling activity which is taking place. When something goes wrong, however, this, too, is immediately apparent. For example, if the diet contains too much fat or carbohydrate, the cell becomes crowded with small fat particles which eventually join together in one great mass, and this mass forces the nucleus to one side and seriously handicaps the normal activities of the cell (figure 2). Or, if the trouble is due to disease, the cell may actually die. The walls shrink and are distorted, and the nucleus is opaque and dark-staining.

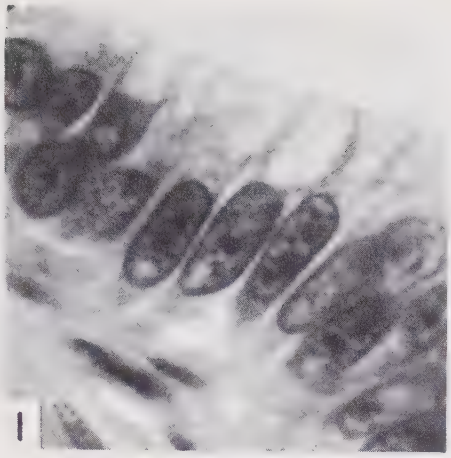
Various nutritional or disease conditions may attack only specific tissues and cells. The effect of a deficiency of pantothenic acid on gills is well known. Other vitamin deficiencies or diseases may affect just the eyes, the nervous system, or the muscles. Usually, the observer

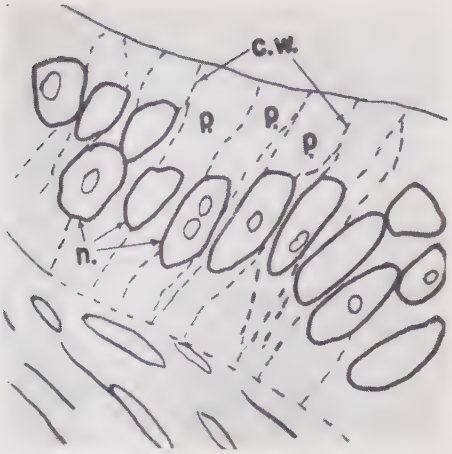
FIGURE 1.-- Normal cells from the intestinal tract of a rainbow trout. x 2000.

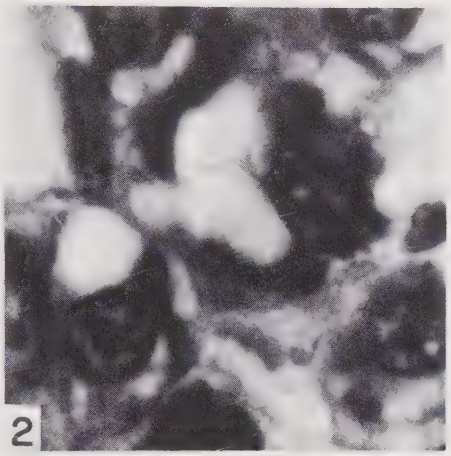
FIGURE 2.-- Fat infiltrating liver cells of a rainbow trout. x 2000.

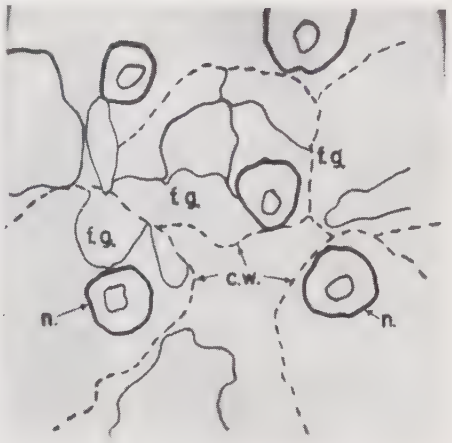
In both figures:

n. - nucleus
p. - protoplasm
c.w. - cell wall
f.g. - fat globule







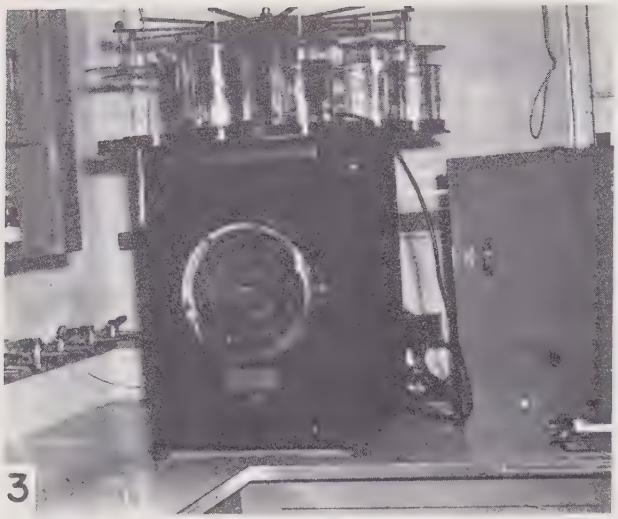


must wait for the development of obvious physical symptoms, such as nervousness, irregular swimming, blindness, slow growth, or death. Even then the chances for correct diagnosis are very poor. Study of the cells themselves, however, makes possible a great step forward in understanding just what is happening to produce the observed symptoms.

The process for preparing tissues for examination under a microscope is time-consuming and tedious. It is fascinating, too, however, for--much like the fisherman who pulls in his seine--the pathologist eagerly waits for the cells to leap into focus so he can examine his catch.

When tissues are removed from a fish, they must be taken care of immediately because the individual cells begin to die at once. The pathologist wants to know whether any of the cells were dead while the tissue was still in the fish. As all will be dead in a very short time, something must be done to stop this process of natural degeneration, which is called autolysis. This is done by placing the tissue in one of several different solutions that are known as fixatives. These solutions coagulate the protoplasm in the cell, the reaction being much like the change which takes place in a boiled egg. This stops the process of autolysis, and living cells then appear alive in contrast to those cells which were dead when the tissue was removed. Thus, the condition of the cells is fixed; hence the term "fixative." This is the reason we urgently ask that fish be slit down the abdomen before being placed in jars of fixative: to allow the solution to get to the inner organs immediately.

In order to examine the tissue on a microscope, it is necessary to slice off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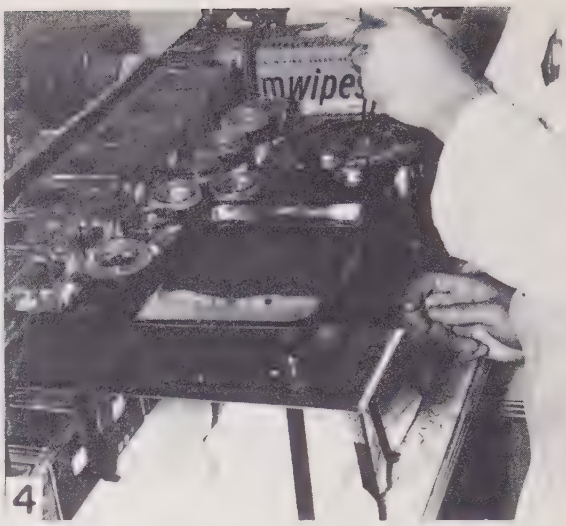

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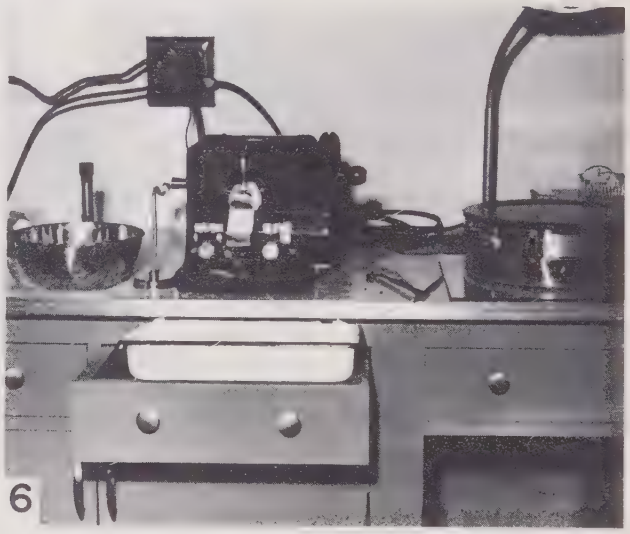
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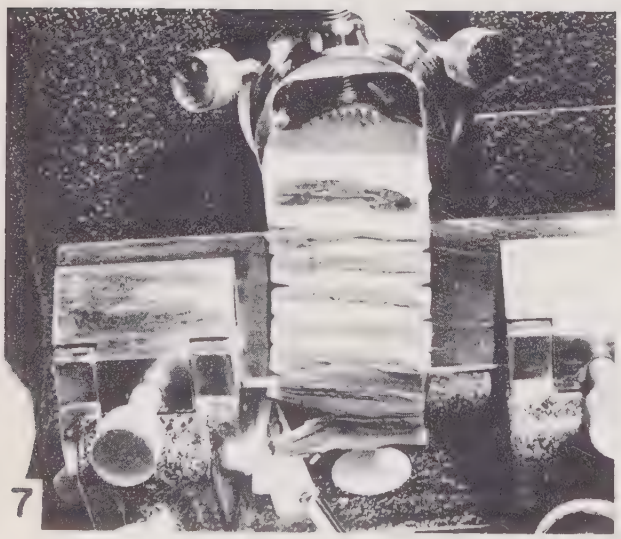
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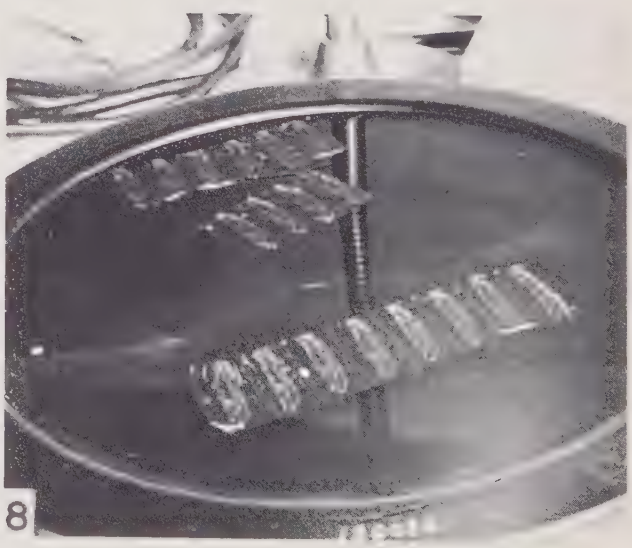
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a very thin piece--a piece so thin, in fact, that light will pass directly through it and into the microscope. The first step is to make the tissue rigid so that it will be possible to cut a more uniform slice. This is done by infiltrating the tissue with melted paraffin and allowing it to harden. First, however, all water must be removed from the tissue, for water and melted paraffin will not mix. To remove the water, the tissue is placed in baths of alcohol of increasing strength or in some other solution which gradually replaces the water. The water must be removed slowly to prevent the cell distortion which would result if the water were removed too rapidly. This slow removal can be effected by transferring the tissue from one beaker to another of fresh solution every 2 to 3 hours and then finally placing the tissue in the melted paraffin. An Autotechnicon (figure 3), however, does the entire process automatically. The tissues are placed in the machine, usually during the late afternoon. They are transferred automatically from one solution to another during the night and are in the melted paraffin the next morning. The tissues are then allowed to solidify in the middle of a small block of paraffin (figures 4 and 5). The microtome is used for cutting the slices or sections. These sections are incredibly thin and are measured in microns. Sections are usually cut from 2 to 10 microns thick, so they are quite delicate. For example, a piece of tissue as thick as the space between 2 words on this page would be cut into about 700 sections. To make cuts of this type, a remarkably sharp knife is required. The cutting edge of the blade is sharpened on special stones or wheels and then checked on the microscope to eliminate even the smallest nick or imperfection. This knife is held rigid in the microtome. The paraffin block with the tissue is held in a clamp. When the handle of the microtome is turned, the paraffin block is forced down over the knife and a section is cut; then the paraffin block returns to its starting position and automatically is advanced the correct distance for the next section. The edges of these sections adhere to each other, forming a long ribbon of sections (figures 6 and 7). Each section is next floated on a bath of warm water, which melts the paraffin just slightly and allows any wrinkles or bumps in the tissue to flatten (figure 8). A glass slide is then placed under the section, and it is removed from the water. A thin, sticky substance has been applied previously; and as the section dries, it is stuck firmly to the slide. The first phase of section preparation is now completed.

To make the section easier to examine, the cells are stained to increase the contrast of their various parts. The most commonly used staining procedure includes hematoxylin and eosin. In this procedure the nucleus is stained a light blue and the other cell parts are stained a bright pink. As each section is encased in a thin layer of paraffin, this must be removed before staining is effective. To remove the paraffin, the slides are placed

(Opposite)

Steps in the histologic processing of a chinook salmon fingerling

FIGURE 3.--Autotechnicon for dehydrating tissues and for paraffin infiltration.

FIGURE 4.--Placing the infiltrated tissues in a pan of melted paraffin for embedding.

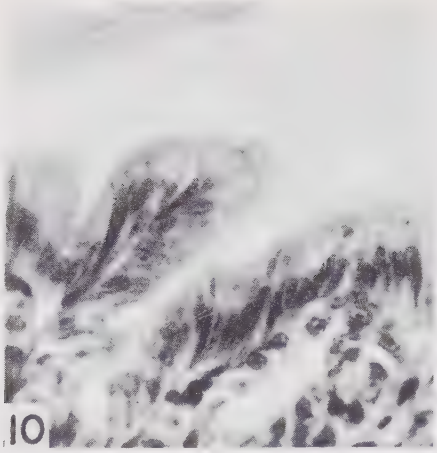
FIGURE 5.--The fish is now embedded in a paraffin block that has been trimmed with electrically heated knife. The complete block of the other tissues is in the background.

FIGURE 6.--The microtome and water bath.

FIGURE 7.--A ribbon of sections extends below the paraffin block over the knife blade.

FIGURE 8.--The sections are spreading and flattening on the water bath.

(Photographs for figures 3 to 8, inclusive, were taken by Mr. Bo Svenonius.)



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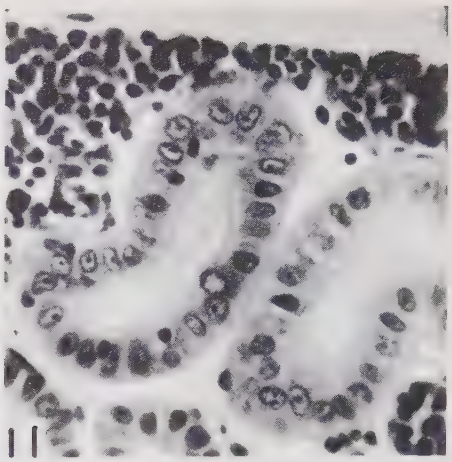

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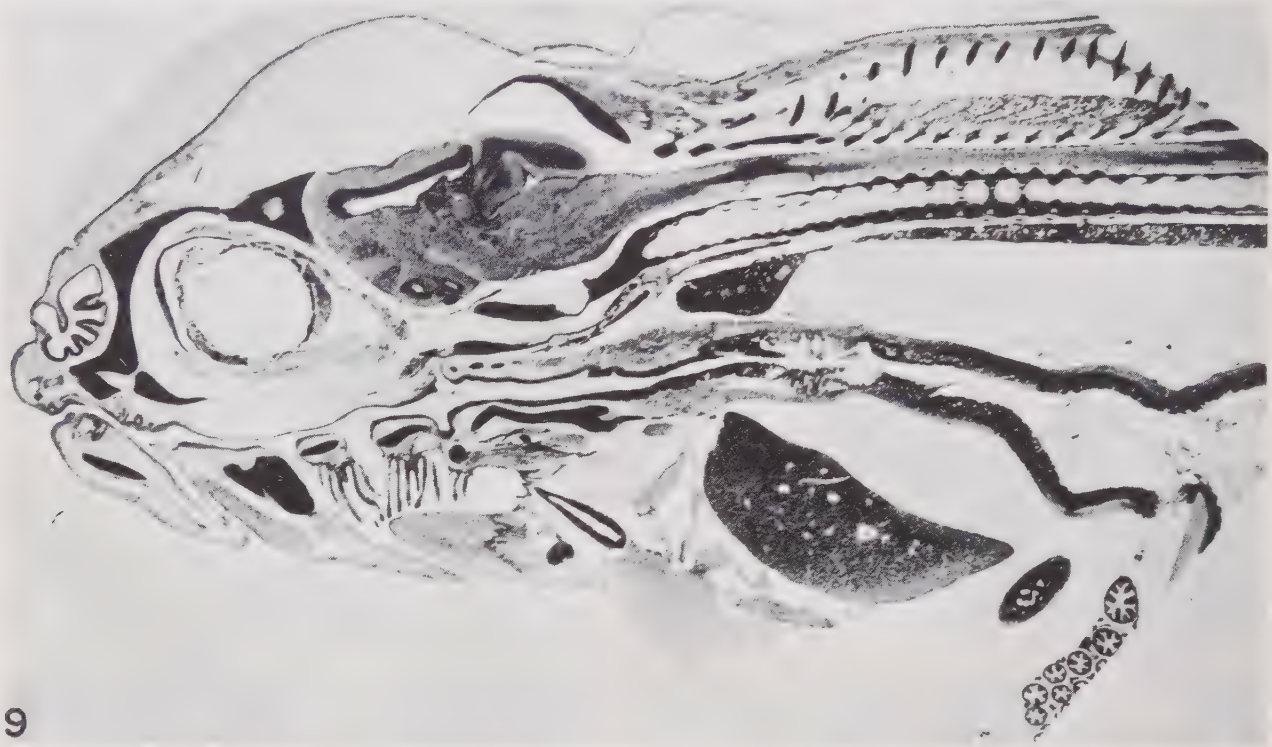

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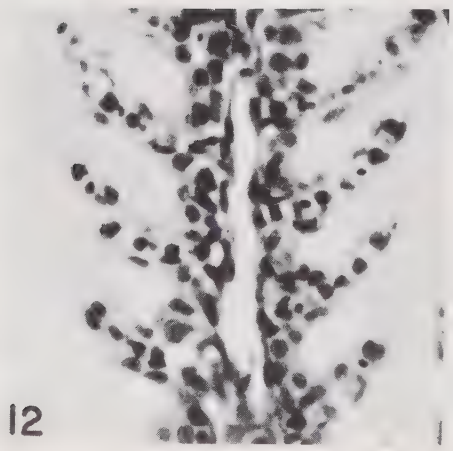

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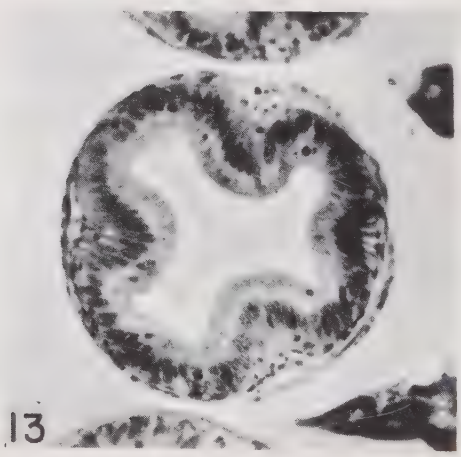

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(Opposite)

FIGURE 9.--The completed longitudinal section of a chinook fingerling, stained with hematoxylin and eosin, ready for microscopic examination. x 4.

FIGURE 10.--The intestinal lining. x 400.

FIGURE 11.--Tubules in the kidney, surrounded by blood-forming (hematopoietic) tissue. x 400.

FIGURE 12.--A gill filament. x 400.

FIGURE 13.--Gastric caeca with adjoining pancreatic tissue. x 200.

in xylene or some similar solvent for a few minutes. The first stain is hematoxylin, which is dissolved in water. It will be recalled that previously the sections were dehydrated to allow the paraffin infiltration. If these sections were placed directly in a solution of water, considerable distortion would result as the water rushed back into the tissue. To prevent this distortion, they are placed first in 95 percent alcohol, then in 80 percent, next in 70 percent, and so forth. Each solution contains a little more water than the preceding one, and the tissues gradually regain their natural content of water. After staining in hematoxylin and eosin, the water must be removed from the sections again. For this purpose the sections are transferred to various solutions of alcohol, each in succession containing slightly less water than the preceding solution. When 100 percent alcohol is reached, all water has been removed. Then the sections are placed briefly in xylene to remove the alcohol, which would otherwise make the sections faintly cloudy. Finally, a drop of clear mounting medium is placed on the section and a thin glass coverslip over that completes the preparation (figures 9 to 13). As soon as the mounting medium hardens, the section is ready for examination.

There are, of course, many special stains which are used to identify specific materials. Bacteria, for example, are often identified by whether or not they stain at all by various techniques. Thus, bacteria may be gram-negative, gram-positive, acid-fast, and so forth. There are specific staining methods for glycogen, blood, iron, hemoglobin, starch, and literally hundreds of other materials.

One additional paragraph will complete this very brief discussion on techniques. A subject of considerable interest in fish pathology is the nature and occurrence of fat in fish tissues. Special methods are required to make fats rigid for sectioning. If imbedded in paraffin, the fat would be removed and lost in the xylene that was used to dissolve the paraffin before staining. A freezing microtome is used for this work. After the tissue is fixed, it is placed on a small platform of the microtome. A jet of carbon dioxide then freezes the tissue solid in a matter of seconds. While the tissue is still rigid, a section is quickly cut. As this section has never been dehydrated, it can be transferred directly to the water solutions of stains and the fatty materials stained. This same type of microtome is used, incidentally, when a very quick diagnosis is required. Thus, when a patient is still on the operating table, the pathologist can examine a tumor and determine whether it is malignant or benign and, consequently, whether or not it should be removed.

In a drainage basin three-fourths as large as the Mississippi Valley, the Caspian Sea receives annually an influx of fresh water estimated to be 175 cubic miles--sufficient to create a lake larger than Lake Erie or to raise the level of the Caspian some 5-1/2 feet--but this enormous volume barely offsets the loss through evaporation.

CHROMOSOME NUMBERS IN TROUT

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IN THE APPLICATION OF GENETICS TO TROUT BREEDING, a knowledge of the chromosomes which bear the hereditary determiners or genes is an important aid. This is particularly so if one is to investigate the possibility of recombination of characters in advanced generation and backcross progenies of species hybrids. The success in obtaining these progenies depends on the degree of fertility of the hybrids. This, in turn, generally is dependent on rather precise association of the chromosomes from one species with those of the other during the processes leading to sperm and egg formation. If the chromosomes from the two species differ markedly in number or kind, high degrees of sterility can be expected.

Preliminary to a study of association of chromosomes in the hybrid gonads, embryonic cells of several species have been studied to determine differences in kind and number of chromosomes in the several species. The present study was also prompted by a desire to compare the observations of Svärdson (1945) on chromosomes of the Swedish Salmonidae to some North American species. Especially of interest was the possibility of shedding light on Svärdson's conclusion that polyploidy has played an important role in species formation in this family.

Ordinarily, a definite number of chromosomes is found in all somatic cells of a given individual and in all individuals of the same species. Therefore, with some exceptions, examination of a few cells of a few individuals should determine the representative chromosome complements of a particular species. Until relatively recently, cytological study of the Salmonidae has been difficult because the fish have large numbers of relatively small chromosomes. Thus, it is not surprising to find inaccurate chromosome numbers reported for species in this family from earlier work (Makino 1951). Recent advances in technique, such as that employed by Svärdson (1945), have removed many of the difficulties. The technique adopted in the present study was a modification of that of Svärdson. Recently, a more elaborate technique has been described by Schreiber (1954).

Materials and Methods

Young embryos of brook, brown, and rainbow trout were used in this study. Eggs of the three species were collected from hatchery egg trays after 2 to 9 days'

This paper was presented at the Conference of the Northeast Section of the Wildlife Society; American Fisheries Society; Fish, Game, and Conservation Commissioners; and the Game Breeders' Association of New Jersey--March 23-25, 1955, Atlantic City.

Publication of the paper was authorized on June 15, 1955, as a joint contribution of the Benner Spring Research Station, the Pennsylvania Fish Commission, Bellefonte, Pennsylvania, and as Contribution No. 195, Department of Botany and Plant Pathology, The Pennsylvania State University, University Park, Pennsylvania.

incubation at 50° Fahrenheit and fixed in a variety of fixatives. These included Bouin's fluid, acetocarmine stain, and Carnoy's fluid, the latter both with and without chloroform. All of these fixatives proved satisfactory. The best results were obtained, however, from eggs that were fixed in acetocarmine for 10 to 30 minutes and transferred to 70 percent ethanol for storage. Fixation time in the other solutions ranged from 12 to 24 hours before storage in alcohol. Good cytological preparations have been obtained after 6 months of storage in this way. Piercing the egg membrane promotes rapid fixation and seems to aid in producing the best preparations.

Eggs collected after 3 to 4 days of incubation gave optimum results under our conditions. At this stage the embryonic cells and nuclei are quite large and the chromosomes spread apart more easily than at later stages. Thus eggs of lake trout and of lake-x-brook hybrids were collected after 20 days of incubation; and although many division figures were found, the chromosomes were packed so tightly in the small nuclei that no determinations were possible. This obviated an important determination, as the above-mentioned hybrid is relatively fertile.

After fixation, the embryo discs were dissected and placed in a drop of acetocarmine on a slide. The embryos were macerated with a steel needle and allowed to stand for a minute or so. Enough iron goes into solution during the use of the steel needle so that no iron need be added to the acetocarmine stain. A cover slip was placed over the material, and sustained pressure was applied to flatten out the tissue. Excess stain was blotted off, and the slide was passed quickly over an alcohol flame several times to intensify staining. Edges of the cover slip were then sealed with paraffin or with a clear fingernail polish. Microscopic examination could proceed immediately, but it was found that staining became intensified upon standing for one to several days. These temporary smear preparations could be stored in a cool place for a period of several weeks without deteriorating.

The preparations were scanned at a magnification of approximately 250 diameters. Chromosomes showed excellent contrast when a green filter was used on the light source. When a suitable figure was located, magnification was increased to approximately 1,150 diameters and a camera lucida tracing was made as an aid in studying the chromosomes. Photomicrographs were made of figures in each species.

Results

In the course of comparing cytological preparations from the many variations in fixation and staining procedures, more than a hundred well-spread metaphase figures were examined. Little can be said yet about kinds of chromosomes except that all three of the species examined had many short rod-shaped ones, and that the species that had the lowest number appeared to have the largest chromosomes. These, as well as some other observations, are in general agreement with those made by Svärdson (1945).

The only previous published report on chromosome number of rainbow trout, Salmo irideus, was that of Behrens in 1898 (cf. Makino 1951). This author reported the somatic number to be 24. In the present study, it has been determined that rainbow trout have 60 chromosomes in each somatic cell. This same number of chromosomes has been found in the closely related Atlantic salmon, Salmo salar (Svärdson 1945, Schreiber 1954). One feature that has been noted in many, though far from all, of the mid-metaphase figures from rainbow trout is the occurrence of one chromosome which appears as an open ring. It is possible that this might be one of a heteromorphic pair of chromosomes associated with sex determination, although sex could not, of course, be determined in these very young embryos.

The somatic chromosome numbers of brook trout, Salvelinus fontinalis, and brown trout, Salmo trutta, taken from

our collection were determined to be 84 and 80, respectively. These determinations conform with the numbers that were reported by Svärdson for these two species. The chromosome morphology in the two species appears to be quite similar. There occurs in each a pair which is much longer than the remainder in the complement, and there are many short rod-shaped ones in each. One of the many brook trout embryos studied had 42 chromosomes in each of its cells. This evidently was a haploid individual that developed from an unfertilized egg. Although this occurrence is rare, it is not unknown in many animals. At least it aided in verifying that the normal diploid chromosome number in brook trout is 84.

Discussion

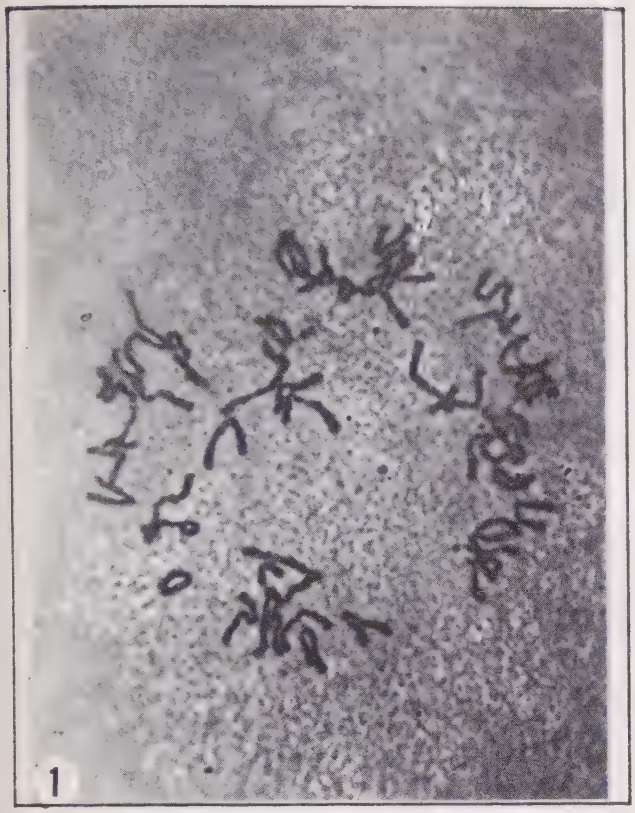
Rainbow trout (2n = 60) and brown trout (2n = 80) have been classified as belonging to the genus Salmo, whereas the brook trout (2n = 84) has been placed in Salvelinus. It is apparent that the chromosome numbers and morphology differ more between the two species of the same genus than between the members of different genera. It has been known for some time, moreover, that hybridization between brown trout and brook trout is feasible, whereas hybridization of rainbow trout with brown trout or brook trout is extremely difficult. On the basis, therefore, of both chromosome-number relationships and ability to hybridize, the species belonging to different genera are more closely related than are the two that have been placed in the same genus.

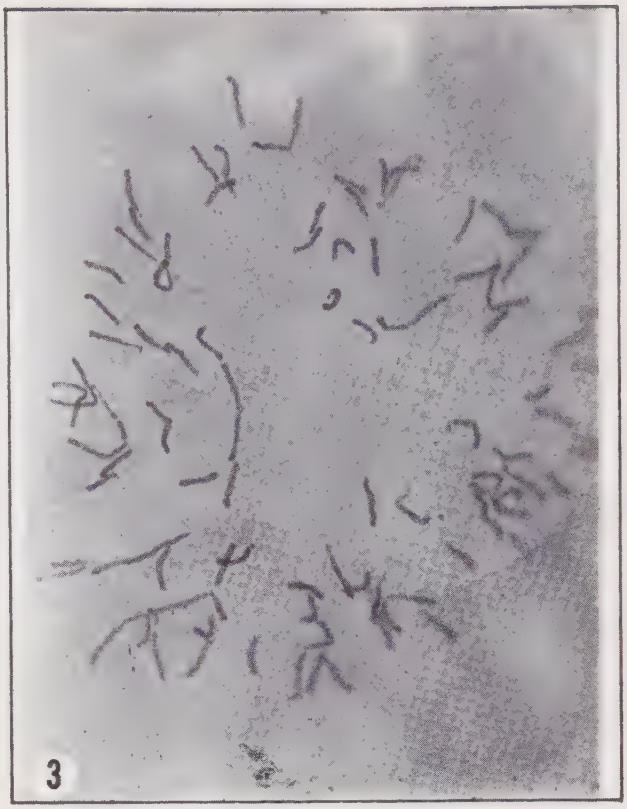
Inability to hybridize infers unbalanced effects of the two very dissimilar chromosome complements from rainbow trout and from brown trout or brook trout. The unbalance could result either through disturbances of normal somatic cell division or through physiological effects of the genes contributed by the two complements. It is possible, however, that these differences are more apparent than real, and that some other factor might prevent hybridization. From preliminary observations at the Benner Spring Research Station, it has been suggested that this factor might be an egg-cytoplasm disturbance related to the extreme differences in incubation periods between the rainbow trout and the brown or brook trout.

Though brown trout can be crossed with brook trout, the hybrids are sterile, probably as a result of more minor differences between the two complements of chromosomes. A study of the association or pairing of these different chromosomes in the hybrids during the specialized type of division leading to sperm and egg formation would better answer the question. From the present study it seems that enough similarity exists between the complements so that some pairing would occur in the hybrids. The production of some functional eggs or sperm might therefore be expected. At least it has been determined that the gonad development in some males of this hybrid approaches the normal in appearance.

On the basis of similarity of numbers and apparent similarity of morphology, one might be able to predict other possible hybrids. Thus, from the information that is now available, it would seem that a cross between the rainbow trout and the Atlantic salmon might produce a fertile hybrid. Both of these species have 60 chromosomes, and in photographs the chromosome morphology of the two species appears similar.

A critical evaluation of the hypothesis of Svärdson (1945) that a polyploid series exists in the Salmonidae is not possible on the basis of these limited results. Svärdson found numbers that ranged from 58 in Osmerus eperlanus, through 60 in Salmo salar, 80 in Salmo trutta, to 102 in Thymallus thymallus. These numbers are approximate multiples of 10, and this was therefore postulated to be the basic number in this family. A more extensive survey of American species would aid in determining whether other numbers that are multiples of 10 exist here.





(Photomicrographs courtesy of the author)

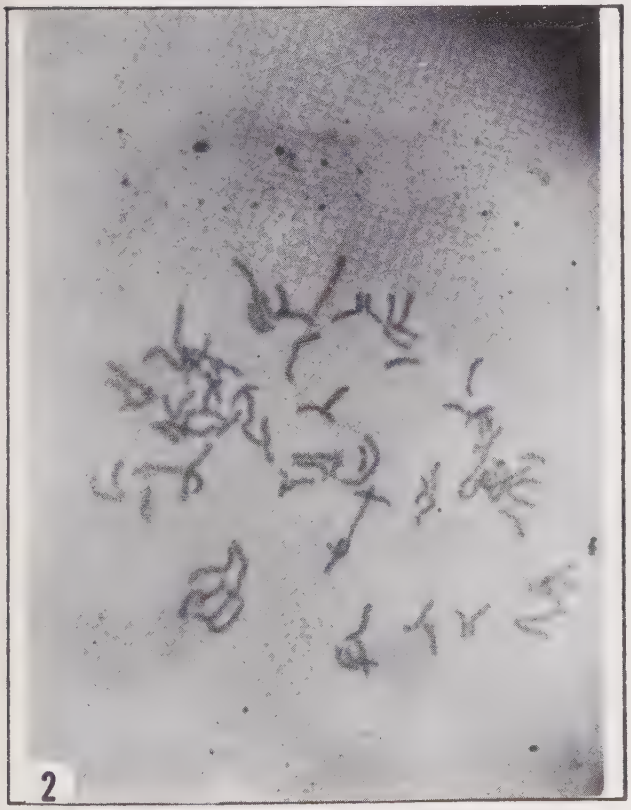


FIGURE 1.--Mitotic metaphase in rainbow trout, Salmo irideus. 2n = 60. Magnification 1400X.

FIGURE 2.--Mitotic metaphase in brown trout, Salmo trutta. 2n = 80. Magnification 1400X.

FIGURE 3.--Mitotic prometaphase in brook trout, Salvelinus fontinalis. 2n = 84. Magnification 1100X.

Many of the cytological preparations that were used in this study were made by Mr. Richard Wahl. Dr. A. R. Grove and Mr. George Gordon aided in the preparation of the photomicrographs.

Literature Cited

Makino, Sajiro.
1951. An atlas of the chromosome numbers in animals. Iowa State College Press. Ames, Iowa. 290 pp.

Schreiber, Jack.
1954. Staining plant and animal chromosomes by the Feulgen-acetocarmine sequence. Stain Tech. 29: 285-291.

Svärdson, Gunnar.
1945. Chromosome studies of Salmonidae. Reports, Swedish State Institute of Fresh Water Fishery Research, Drottningholm.

A DEFOAMER USED TO PREVENT FOAMING IN FISH TRANSPORT TANKS

The appearance of foam and scum when fish have been put into a tank for transport presents a problem that is familiar to most hatchery workers. In California, however, where drugs are used during the movement of fish by truck or by airplane, the effects of foaming have been regarded as particularly detrimental to the carrying condition of the fish. Here, aside from causing a loss of water, the formation of foam decreases the concentration of the drug and thereby lowers its effectiveness.

John E. Skinner of the California Fish and Game Department undertook to determine the desirability of using a defoamer in the tanks. The results of Mr. Skinner's work are reported, in part, as follows:

> ... Dow Corning Antifoam AF Emulsion ... may be extremely useful in transporting catchable trout by preventing the accumulation of foam and scum in planting tanks. ...
>
> The product under consideration completely prevented any foam formation in a 150-gallon test tank to which had been added 1 grain of sodium amytal per gallon of water (100 gallons) and 100 pounds of rainbow trout (10 trout per pound). In another test with the same size of tank, 25 cubic centimeters of methyl pentynol, an especially heavy foamer, was added to 100 gallons of water. Two hundred pounds of trout were loaded into the tank to establish a good accumulation of foam and scum. After 45 minutes, when the foam was rolling from the top of the tank, 25 cubic centimeters of a 10-percent dilution of the antifoam emulsion was added. Within 1 minute all foam was eliminated and only a thin film of scum remained.
>
> About 50 cubic centimeters of the 10-percent solution was adequate to stop all foaming with as much as 75 cubic centimeters of methyl pentynol in 100 gallons of water. With the obvious success of this product in preventing the formation of foam and scum in these tests, further tests were deemed unnecessary.
>
> There were no apparent toxic effects, although the fish are being held under observation. ... As a matter of fact, the emulsion is used in the preparation of foods.
>
> At present it appears that the substance has a wide latitude of effectiveness and therefore should be relatively easy to use.
>
> The remaining question of concern is the longevity of this compound en route. At this time it appears that 25 cubic centimeters of the 10-percent dilution will be adequate for an 8-hour trip in the 150-gallon transport tank and proportional increases in the larger tanks, estimated at about 25 cubic centimeters (approximately 1 fluid ounce) of the 10-percent dilution for every 100 gallons of water. ...
>
> The elimination of foaming should permit the retention of effective concentrations of drug by preventing their loss through foaming. ...

Mr. Skinner also notes that a defoamer can eliminate the added labor of cleaning scum and foam dried on tank surfaces.

WINTER SURVIVAL OF HATCHERY-REARED AND NATIVE BROOK TROUT

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East Lansing, Michigan

THE PROBLEM OF THE RELATIVE INABILITY OF HATCHERY-REARED FISH TO SURVIVE THE WINTER has been a major concern of biologists and fish culturists alike. The methodologies employed in assessing the importance of this variable have generally been of two related types. The first consists of planting fish late in the fall and doing a population estimate in the area of plant, immediately prior to the opening of fishing season, by the employment of one of the various shock methods. This type of study has been summarized by the Wisconsin Department of Conservation (1953). The second method usually employed is that of planting the fish in the fall and estimating relative abilities to survive on the basis of results from creel-census reports (Smith and Smith 1945), voluntary reports of fishermen (Nesbit and Kitson 1937), and random creel-census reports (Heacox 1944) during the open season. The inferences and conclusions drawn from such studies have provided overwhelming evidence that hatchery-reared fish have a uniformly low rate of survival and are vastly inferior, in general, to natives of the same species.

However, in the absence of some restraint on movement, it is very likely that hatchery-reared fish would seek a placid environment similar to that presented in typical hatcheries. Therefore, when population checks by the two aforementioned methods are made, the results are necessarily dependent upon the number of fish remaining in the area of plant. It would follow from the above that any attempts to determine the relationship of winter weather to ability to survive must involve the placement of fish in a confined area and thereby insure the representativeness of the later sampling. That this variable was not controlled adequately in the past is a serious omission and is generally a flagrant violation of principles of scientific research.

Whether the second method cited for studying survival is adequate without reservation even in screened-off areas is, in reality, an empirical question. The use of number of creeled fish as an index of survival involves logical jumps and assumptions which cannot be backed by the logical tools used by contemporary philosophers of science. Any inference to population survival from creel reports must necessarily involve both screened-off areas and determinations of remaining numbers by reducing water level and shocking. It is the conclusion of this study that creel methods for studying mortality or survival are impracticable and, where employed, must be conjoined with the control of the two aforenamed variables. Only through this method can adequate scientific inferences concerning survival be drawn.

Psychological Research Services, Inc., is under contract to the Michigan Department of Conservation.

A further serious lack of control is found in the plant itself. Prior to plant, the native population with which comparisons are to be made is surveyed roughly; total numbers therefore represent only an approximation of the total number of fish present. Into these waters are planted very accurate numbers of hatchery fish. Therefore, percentage comparisons between hatchery and native fish represent an accurate figure on the one hand and an approximation, at best, on the other. Thus, any conclusions based on these comparisons are strongly suspect and no more accurate than the methodology employed.

The purpose of the present study, then, was to compare the effect of winter months upon the rate of survival of both hatchery-raised trout [1] and native trout. In general, the procedure was to plant brook trout of both types of rearing into two streams with screened-off areas so that the fish could not radically change their location. Monthly population determinations were made by utilization of a 240-volt direct-current shocker. In this manner the relative survival rates could be determined for 30-day periods; and if a split in survival between the two species were forthcoming, that point of bifurcation could be exactly determined.

Procedure

The two sites chosen for the present study were Diversion Sections I-A and II-A, at the Hunt Creek Experimental Station, Montmorency County, Michigan, and Slagle Creek, Wexford County, Michigan. The Hunt Creek area is 570 feet in length, contains no deep pools, but is provided with artificial cover. Slagle Creek, on the other hand, is approximately 1,500 feet in length, contains several deep pools, and has an abundance of natural cover. At both these sites screens or dams at the upper and lower ends prevented escape from the enclosed areas.

Six weeks prior to the initial plant, 190 brook trout were marked by the fin-clip method. The early marking date was selected to preclude the possibility of mortality due to anesthetization and clipping at the time of planting. The mean length in the group was 7.67 inches, and the mean weight was 0.183 pound per fish.

Because of the unavailability of storage space for lengthy periods, the natives used in the present study were selected, marked, and introduced into their respective areas within the 2-day period preceding the total plant. All fish selected were of the same mean weight and length as the hatchery controls. The natives used in these Hunt Creek diversion sites were secured through shocking of the North Branch of the Au Sable River, Crawford County, Michigan. One hundred and two fish were used, the extra animals being used to insure representative numbers in the event that some mortality due to transportation should occur. However, a close daily check of this planting site revealed no loss due to transporting the fish from their native habitat to the planting site.

The native brook trout that were used at the Slagle Creek area were indigenous to that stream. The areas utilized were shocked initially, and 35 fish of the same average weight and length as the controls were retained as experimental animals, while the remaining fish were transferred below the dam. By employment of the above methodologies, the effect of transportation on the ability to survive of native fish could be studied, because at one site the fish were transported and at the other they were native to the stream.

On August 26, 1953, the hatchery controls were planted at their respective sites. One hundred fish were introduced into Hunt Creek diversion area, whereas 90 fish were utilized in the Slagle Creek area. Other studies being run concomitantly with the present study at the same sites resulted in an overpopulation [2] of

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[1] Hatchery trout should not be confused with "trained" trout, which are not being reported in this study.

[2] Six times the normal population were planted at Hunt Creek; three times the normal population were used in Slagle Creek.

the two streams used. It must be remembered, then, that the results presented herein represent deflated values at best and must not be construed to represent absolute rates of survival under normal conditions. Rather, the results represent rates of survival under poorest possible conditions and may be used for comparative purposes only.

Results

The results of the major part of the present experiment are shown in table 1. As can be seen from a close inspection of the data, the areas that were utilized produced widely disparate results. In Hunt Creek the final shocking revealed that the natives were surviving at a rate of 59 percent and the hatchery fish at a rate of 28 percent, whereas at Slagle the figures are 37 percent for the natives and 64 percent for the hatchery trout. Statistical tests on these rates show the differences to be significant beyond the 1 percent level of confidence (Chi square = 19.54 for the former and 7.01 for the latter).

At Hunt Creek the largest drop in percentage survival for trout of both types of origin occurred sometime during the first 30 days after planting. However, differential survival rates first became apparent during the 30-day period between January and February. The initial rapid drop may possibly be due to angler encroachment, as the diversion sections utilized were easily accessible to trespassers and fishing was permitted less than 30 feet from this survival site.

In the Slagle Creek area the 2 groups of fish showed their most rapid drop at different points during the 6-month period. The native fish dropped 20 percent in the 30-day period extending from January to February, and the hatchery fish dropped 13 percent during the first 30 days. As this area is relatively inaccessible to encroachment, it is felt that the results shown here more accurately represent survival figures extant and are not confounded by the operation of other uncontrolled variables.

Discussion

The widely disparate results presented here support one main conclusion. When survival is studied in controlled areas with monthly population determinations, there is no evidence for increased rates of survival as a function of origin of the fish studied.

The determination of the specific factors which contribute to depressed survival rates are difficult to ascribe. The fact that at the Slagle area the fish were in competition with rainbow trout, while at Hunt Creek they were not, would tend to withdraw support from the contention that competition is of great importance to survival. Recent starvation studies (Adelman, Bingham, and Maatch, 1955) have revealed that this variable could not be a contributing factor. Whatever the cause, there seems to be little or no difference between hatchery-reared brook trout and native brook trout in their ability to survive the winter months.

TABLE 1.--Percentage survival of hatchery and native brook trout as determined by monthly population checks

	Percentage survival on--					
	Sept. 21	Oct. 27	Nov. 30	Jan. 7	Feb. 1	Mar. 1
Hunt Creek Diversion:						
Hatchery brook trout----	66	73	64	64	35	28
Native brook trout------	86	79	67	72	65	59
Slagle Creek Area:						
Hatchery brook trout----	87	85	75	74	64	64
Native brook trout------	83	75	75	63	43	37

Literature Cited

Adelman, H. M., J. L. Bingham, and J. L. Maatch.
1955. The effect of starvation upon brook trout of three sizes. Prog. Fish-Cult. 17 (3): 110-112.

Nesbit, R. A., and J. A. Kitson.
1937. Some results of trout tagging in Massachusetts. Copeia 1937 (3): 168-172.

Heacox, Cecil.
1944. Stocking experiments on Wiscoy and East Koy Creeks. The Empire State Sportsman 4 (7).

Smith, L. L., Jr., and B. S. Smith.
1945. Survival of seven- to ten-inch planted trout in two Minnesota streams. Trans. Am. Fish. Soc. 73 (1943): 108-116.

Wisconsin Conservation Department.
1953. Wisconsin trout management. 19 pp., illus. Processed.

PROGRAM FOR THE MUSSEL

The competition from plastic materials and imported marine pearl shells has given the domestic mussel-shell industry a difficult time in recent years. During 1954, the industry produced 4.3 million gross of buttons, valued at $4,800,000, and 3,155 tons of lime and poultry grit, valued at $22,000. The value of manufactured items produced from fresh-water mussel shells averaged $4,900,000 in the last 3 years, whereas during the period 1936-38 this value averaged $4,100,000. Nevertheless, this gain in value has been due entirely to the increase in prices for buttons, as the quantity produced has declined substantially during the period.

Most of the mussel shells taken in this country for manufacture into fresh-water pearl buttons have been produced in waters of the Mississippi River basin, especially in areas of the Tennessee River. Thus, during 1954, Tennessee accounted for 30 percent of the total take of mussel shells; Alabama, 20 percent; Arkansas, 18 percent; Kentucky, 13 percent; Indiana, 11 percent; Illinois, 7 percent; and Iowa--center of manufacture--only 1 percent.

At this time there is no demand for shells taken from the northern rivers outside the Mississippi River basin. The principal species taken from these waters is the mucket or "pocket book" mussel, the shell of which is less suitable for the type of button now desired.

Of greatest importance to the domestic button industry are the niggerhead and the pigtoe, mussels that have a comparatively heavy shell. A program of investigation designed for improvement of the mussel resource will include life-history studies on both these species, as well as a study to determine the host fish of the pigtoe.

It is obvious, however, that more than life-history studies will be necessary to reverse the recent trend in mussel populations. Consequently, present plans of the U. S. Fish and Wildlife Service for this work include also: a survey of the TVA area of the Tennessee River to determine the shell potential, particularly as applied to juvenile populations; a study of the effect of siltation in the TVA system on the mussel populations, particularly on the juvenile sizes; a survey to locate new mussel beds; a study of regulations on the harvesting of mussels; a study of the possibility of improving present fishing gear and other methods of shell harvest; and a study of the possibility of transplanting desirable heavy-shell mussels to areas where desirable populations do not now exist but where the biological conditions might permit the establishment of such populations.

THE EVOLUTION OF A REARING PROGRAM IN WYOMING

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IN WYOMING, as I presume is true in nearly every other State, we have been faced with what appears to be a never-ending increase in the number of fishermen, coupled with a continued loss of suitable fishing waters because of increased land and water use, growth of population, and pollution. In some instances, environmental changes have been so great that certain waters must depend on artificial restocking as a means of meeting the demand, even though it is realized that stocking alone cannot be depended upon to be the panacea for all the related troubles. In order for fish to be available as "catchable-size" fish in the minimum length of time, it is necessary that they be planted at a comparatively larger size than ordinarily has been used for maintenance-type planting. At the same time, our Department is attempting to plant fish in larger numbers to meet the demand of increased fishing pressure and to insure proper and sufficient distribution.

Although figures of the plantings of small fish look good on paper, actually the percentages of survival of such fish after they are introduced into the various streams have proved to be rather small--at best around 30 percent and sometimes as low as 5 percent.

Everything, therefore, seemed to indicate the inauguration of a plan whereby we could raise large quantities of fish, yet raise them to a comparatively larger size. It was felt that the existing hatcheries were able to hatch sufficient eggs to supply all our needs; and, with the expansion of the facilities at each station, we were able to raise these large numbers of newly hatched fish to small fingerlings and to raise a limited number of larger fish. The bottleneck came in raising large numbers of fish to a more suitable planting size (6 inches or over). In order to rear fish to a larger size, it is, of course, necessary to have more space available in which to grade them progressively as they grow larger, and they must be raised under conditions somewhat different from the hatchery troughs which are sufficient to maintain fish of small size.

Establishing a program of this kind requires that there be sufficient rearing facilities available to carry the increased load. The rearing facilities at all our hatcheries had been enlarged to make possible the rearing of fish in larger numbers, but there was still a need for facilities beyond those which our hatcheries could furnish. Real facilities can be constructed, but their operation is impractical unless they are accompanied by additional water supplies. At existing stations the situation soon reached the point where further expansion was out of the question. (In Wyoming, springs that flow more than 3 cubic feet per second are considered large.) The cost of developing such a program at the hatcheries appeared to be far in excess of what our limited budgets could stand. A means was sought wherein fish could be reared to a suitable planting size at some point removed from the hatcheries without entailing too great an outlay of funds.

It followed almost naturally, in view of the fine fish that were being taken from various natural ponds and farmers' ponds,

that the solution was in using such ponds scattered throughout the State as a means of supplying the much-needed larger fish. The plan was to plant 2-inch fish from the hatcheries into the ponds in the spring and remove them from the ponds the following fall or spring as 6- to 8-inch fish which had grown to that size on natural feed and under natural conditions. Unfortunately, this sounded better than it proved to be when put in operation.

A definite program, in which ponds or pond sites to be used were secured under lease agreements with the landowners, was outlined and put in operation. Money was appropriated for rearing-pond construction and maintenance, and sportsmen's clubs were urged to join in the program and to aid in preserving good fishing by sponsoring ponds of their own. In many cases the program was readily accepted by sportsmen, and they contributed much of their own time and money in getting their favorite projects completed. It proved to be an excellent way of getting new members for their clubs, because it could be shown that here was a project upon which the Game and Fish Commission had bestowed its blessings and in which the sportsmen were able to take an active part. As far as the Department was concerned, we were receiving some much-needed assistance in getting a big job done in the shortest possible time and, at the same time, affording a means whereby the sportsmen could profitably expend their efforts in a controlled and meritorious project.

There was established a definite standard for a pond which was to be used for rearing purposes. Included were the following requirements:

1. The soil on which the pond is built should have a fairly high clay content so that the pond and dam will hold water and so that, when drained, the pond will not become a veritable bog.
2. The water source should have a temperature between 50° and 65°, with a flow of at least 60 gallons per minute per acre of pond.
3. The surrounding area should not contain heavy deposits of coal, sulphates, or other minerals which would be detrimental to fish if dissolved or suspended in the water.
4. Large drainage areas should be avoided in the selection of a site, as they carry excessive amounts of run-off water. Sites utilizing spring water would be preferable, as they afford more constant flow and temperatures.
5. One-third of the pond should be at least 8 feet deep to prevent freezing to the bottom in winter and to prevent the water from becoming too warm during the summer.
6. The sides of the pond should drop off sharply to keep herons and other wading birds from preying on the fingerlings. Some cover is desirable.
7. Rearing ponds should be accessible at all times of the year, and--so that long hauls for stocking will not be necessary--the pond should be located near the waters it is to service.
8. The good rearing pond should have a controlled outlet, such as a valve or dropboards, that will permit rapid drainage and control of water flow.
9. Ponds that are smaller than 1 acre should be considered too small to be practical for rearing purposes.
10. Ponds used for rearing purposes should be closed to fishing.

With all these criteria, it would seem that our problem was well on the way to being solved. We found, however, that we had many things to learn about the operation of rearing ponds away from the hatcheries. The first was that the two were not comparable in either operation or production. Even when built according to definite specifications, ponds will differ greatly in production and in suitability for various types of management. Here we were dealing entirely with trout production. We found that in some ponds where there were no other species present trout would thrive under physical and chemical conditions which were previously thought unfavorable, or even intolerable, for them. Ponds

which were fed directly or indirectly from streams often contained minnows, suckers, and other stream fishes; but if the water was cold and well aerated and had an adequate flow, trout seemed to withstand the competition of other species. However, any competition in a borderline pond usually proved disastrous to the trout.

In our operations there always seemed to be one thing or another which prevented realization of the kind of production that should be expected for practical operation. Some of this can be attributed to the typical trend in virgin lakes or artificial impoundments, where after the first 4 or 5 years the productivity often starts on a gradual downward slide. In spite of the operation of what appeared to be a good program, the fishing was showing no particular improvement even after the program had been in effect for several years. As the ponds had been built here, there, and everywhere throughout the State--and many were literally a hundred miles from any place--we figured that the failure must be due to lack of proper management and control. At that time we began keeping definite records on each pond and set up a rearing-pond crew to operate and maintain the ponds. From these records, we believe, we are now able to prove the impracticability of the outlying rearing ponds by revealing what a small percentage of fish they actually make available for the fisherman.

Rearing-pond records were set up in ledger form just like a set of books: they showed the number and weight of fish that went into the pond each year and the number and weight of those that came out each year. In addition, we endeavored to ascertain whether the ponds were paying for themselves: we placed a value on the fish when they were planted and compared that with the value of the fish recovered from the pond, less the expense charged against maintenance of the pond. For lack of something better, the figures used were the commercial values of fish of the sizes being dealt with, as follows: 1- to 2-inch fingerlings at $3 per hundred, 3- to 4-inch fingerlings at $7.50 per hundred, and 5- to 6-inch fingerlings at $15 per hundred.

We planned, at first, to designate only those ponds that gave at least 50 percent survival as good ponds, worth keeping in operation. We found, however, that no ponds were that successful. When we tried to determine which of the ponds were breaking even, from a monetary standpoint, we found that most of them were badly in the red. We had previously decided that if a pond just broke even, it was worthy of operation, especially in view of the intangible values to be derived from the availability of larger fish for stocking purposes. Throughout a period of several years, we endeavored to weed out the poorer ponds and operated only the better ones. In 2 years we had reduced the number of ponds in operation from 136 to 98 and had attempted to give those in operation all the care we possibly could. Even so, we found that 90 of the 98 ponds were operating in the red. In other words, the number and size of the fish being made available for planting were not enough to make the operation practical.

In addition, the percentage of survival was very poor when all the ponds in the State were considered. It is true that a few each year showed good production, but in each case this production could be attributed to some special attention or artificial feeding by hatchery personnel, sportsmen's clubs, or an interested landowner. Survival figures were secured by using the percentage of fish planted from the pond as compared to the number of fish planted in the pond. During the years that this program was in operation, the results--even though we tried to operate only the best ponds--were as follows:

Year	In	Out	Percentage survival
1st	767,860	158,267	20.6
2d	1,123,173	218,925	19.5
3d	1,230,392	149,390	12.1
4th	1,200,210	114,183	9.5
5th	675,315	100,208	14.8
6th	582,426	56,216	9.7
7th	525,738	78,467	14.9
8th	400,222	46,370	11.5

During the second year, for example, the 1,123,173 placed in the ponds averaged 1.6 inches in length and the 218,925 fish recovered averaged 6.1 inches.

A record of this kind called for immediate inventory of the causative factors for such a trend. They were found to be many. Efforts were made to develop some formula from which could be derived a numerical coefficient that would indicate whether any one pond was good, bad, or indifferent, as compared to all the others. There seemed to be too many factors involved for inclusion in any one formula, and their interaction was not in any way constant. Further, the lack of definite control appeared to be the cause for most of the trouble or, at least, was a contributing factor in all cases. The very nature of the pond locations and the fact that they were so widely separated precluded having personnel available to look after them throughout the year; and unless this can be done, there is always something which can be depended upon to make the operation of any individual pond a failure. These occurrences may include: washing-out of a dam or spillway by excessive runoffs or flash floods, a condition which can be prevented if someone is present to adjust the gates; mossing-over of a screen, causing the water to back up and eventually to flow over a dam that is not equipped with a spillway and take fish with it, or to wash out the dam; use of the waters for irrigation purposes before it is anticipated, thus causing premature drainage of the pond or lowering the water level beyond the point where the fish can survive; heavy predation by herons, mergansers, and other predators; winter kill in ponds where fish are held for a year; outright vandalism; fishing by unscrupulous fishermen; beaver-dam building activity, which either plugs the outlets or closes the inlets; muskrat activity in dams, causing loss of water; unscreened outlets or spillways; water conditions not analyzed sufficiently beforehand and later found to be unproductive or of undesirable temperature; water-supply source cut off for some reason; large fish which enter through the water supply and eat the smaller fish; incomplete drainage of ponds in previous years, causing newly planted fish to serve merely as forage for large fish left in the pond from years before; or excessive amounts of silt in the ponds.

There were instances where fish in the ponds actually showed excellent growth and survival yet never reached the water to be planted because they were stranded in heavy aquatic growth in the pond during its drainage--or the lowering of the water level might cause the water to become too warm or too gaseous and thus kill the fish before they could be removed.

Another factor contributing to poor production records was the frequent overstocking of the ponds. The pond area usually was not enough to maintain the excessive number of fish, especially as they grew larger. In other words, the value of the fish put into some ponds was so great that it could not be offset even by high production of the pond. Cannibalism was, of course, prevalent under these conditions. During the past few years we established a policy of rearing-pond planting on the basis of 5,000 2-inch trout per surface acre of water, with some improvement in results.

Our rearing-pond program also showed us what can happen when conservation departments solicit the aid of sportsmen's organizations. We were firmly convinced that increased rearing facilities would supply the desired improvement in fishing conditions, and we sold the clubs throughout the State on the idea--too thoroughly, perhaps, as later we experienced some difficulty in reversing the process. It is true that many organizations gave unstintingly of their time and funds in furthering the program, but many times it proved to be better as a means of showing prospective members how active the organization was--and how well the organization was cooperating with Department programs--than as a means of improving fishing conditions. Records from ponds not under Department control were not always so accurate as could be desired. A few large fish seen in a pond might result in good production reports when, in reality, production was very low. Many production

estimates were derived from the number of fish planted as compared to anticipated production, rather than from actual observations of the fish removed. Production records were padded so that larger numbers of fish would be stocked in a particular pond; and applications were sometimes made for large numbers of fish, only some of which were put in the ponds. Exact areas of ponds often were not known by the sponsoring club and were exaggerated purposely so that the club would receive more fish for planting; consequently, the output records of such ponds also had to be exaggerated. Sportsmen sometimes lost sight of the real purpose of the ponds--namely, to raise small fish to a more desirable planting size--and were particularly disgruntled when fish 6 inches long were not planted in their ponds in the first place. Another difficulty arose from the fact that sportsmen felt that the fish from any pond which they had sponsored should be planted according to their dictates, regardless of any Department plans or policies.

Obviously, something had to be done to correct the situation. At first thought it appeared that the best thing to do would be to divorce ourselves completely and as rapidly as possible from all rearing ponds and their manifest problems. However, it was realized that the need for adequate rearing facilities still existed. The problem was to develop some more efficient rearing system with which to replace the rearing ponds. As lack of definite control was known to be one of the chief factors affecting rearing-pond production, it was indicated that a controlled project which embodied the fundamental principles of the rearing-pond program would be the logical solution. It was known that the scattered locations and the distances between ponds were factors that made control difficult; so it naturally followed that if a group of ponds having sufficient output could be located at one place, putting a permanent caretaker in charge of them would be the practical procedure. Thus was born the current plan of developing rearing stations where suitable sites are available.

Results from the three rearing stations since put in operation in Wyoming have far exceeded our fondest hopes. The survival has exceeded 85 percent over a year; and because it is practical under these conditions to feed the fish artificially, the time from when they leave the hatchery until they are large enough for planting is considerably reduced. Although the initial cost is greater than that of a number of rearing ponds, the output and survival percentage will soon pay for the difference, and each station can actually replace many of the outlying ponds.

Our present program calls for hatching eggs and rearing fish at the hatcheries to approximately 2 inches, after which the rearing stations supplement the output of several hatcheries in the surrounding area by assisting with the rearing of these fish to a size more suitable for planting. Both ponds and raceways are used at the rearing stations, and permanently assigned personnel keep the operations on a sound and efficient basis. Only four of the original outlying ponds are being used now, and these are close enough to hatcheries to be utilized as part of their normal operations.

Many of the former rearing ponds are now being utilized in the rapidly expanding farm-pond program. Our prerequisites for good rearing ponds lend themselves well to the development of farm ponds. We are not attempting to replace hatcheries with rearing stations, for we realize the need for both in our program; neither are we attempting to go into an all-out planting program of catchable-size fish only. In Wyoming, at least, we feel that there is need for planting both fingerling and adult fish. Small fish are more practical in the planting of rehabilitated lakes, virgin waters, and those lakes where a good percentage of survival is assured; the larger fish are, of course, most desirable where the waters are readily accessible and extremely heavily fished or where they are unable to support a resident population. The planting of large fish which are caught immediately thereafter does not make for fishing conditions that are practical in all situations. Every attempt is made to enhance the natural conditions that will keep fishing in the realm of a sporting recreation based on wild fish.

DISPERSAL OF STOCKED NORTHERN PIKE IN CLEAR LAKE, IOWA

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IN THE FALL OF 1953, 2,630 tagged young-of-the-year northern pike, Esox lucius L., were stocked at 8 locations in Clear Lake (figure 1, table 1). The tag returns give an opportunity to test the hypothesis that the stocked pike are caught predominantly in the area stocked.

Clear Lake, in north-central Iowa, is a shallow lake with an area of 3,643 acres and a maximum depth of approximately 20 feet. The length and width are approximately 5 and 2 miles, respectively. There are no important inlets or outlets into which pike migrate except Ventura Marsh, which has a trap where all migrating pike may be counted.

A relatively small resident population of pike was in the lake prior to the fall of 1953. Intensive gill-netting caught comparatively few pike from 1947 to 1952 (Carlander 1953). The annual average catch per day per 125 feet of experimental gill net ranged from 0 to 0.3 pike, as compared to 4.4 to 18.0 walleyes. In 1954, the average catch was about 1.3 pike and 6.3 walleyes per gill-net day. In October 1953, 15,192 young pike, mostly 10.5 to 16.0 inches in total length, were stocked in the lake. Of these fish, 2,630, or 17 percent, were individually numbered with monel-metal strap tags around the premaxilla. Anglers caught more pike in the summer of 1954 than in any previous year and reported on 780 of the tagged fish. The locations at which the pike were caught by the anglers were recorded for 651 of the tag returns. As more than 6 months had elapsed since the fish were stocked, it was felt that they had had time to distribute themselves to suitable habitats in the lake.

The lake was divided into seven areas (figure 1) for evaluation of the tag returns. Insofar as possible, the seven areas were selected to provide homogeneous environments. Some of these areas appeared to provide better habitat for pike than others, and it was not expected that the pike would be equally distributed among the areas. The tag returns indicate that most of the pike were caught from certain areas (table 1, totals).

Regardless of where the pike were stocked, they moved around and were caught from all areas of the lake (figures 2 to 8). If the distribution of all the tag returns is considered as representative of the distribution of the pike population, it is possible to test to see whether the pike from each of the plantings show a similar distribution (Chi-square = 65.6 with 36 degrees of freedom, which would occur less than 1 time in 100 if each of the plantings of pike became equally distributed). There is a tendency for more of the fish from some plantings to be caught in certain areas than for fish from other plantings.

From Project 39 of the Iowa Cooperative Fisheries Research Unit, sponsored by the Iowa State Conservation Commission and the Industrial Science Research Institute of Iowa State College, with the cooperation of the U. S. Fish and Wildlife Service.

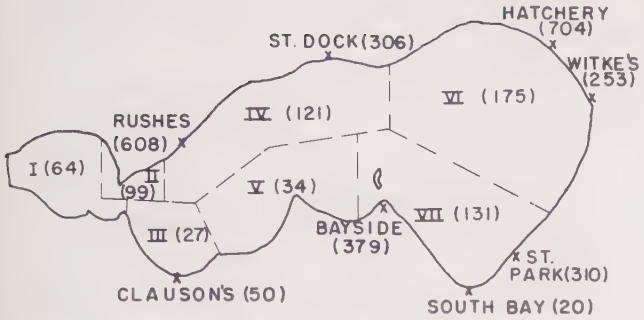

FIGURE 1. NUMBERS OF TAGGED PIKE RELEASED AT THE 8 STOCKING LOCATIONS AND THE NUMBERS RECAPTURED IN EACH OF 7 AREAS.

FIGURE 2. RECAPTURES OF TAGGED PIKE RELEASED AT CLAUSON'S COVE.

FIGURE 3. RECAPTURES OF TAGGED PIKE RELEASED AT THE RUSHES.

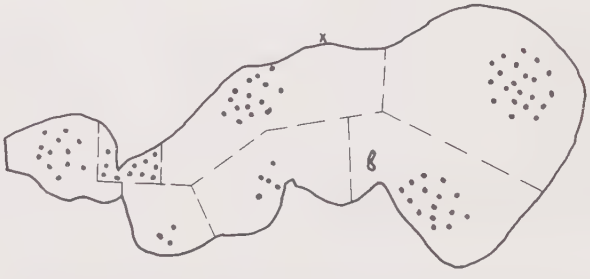

FIGURE 4. RECAPTURES OF TAGGED PIKE RELEASED AT STATE DOCK.

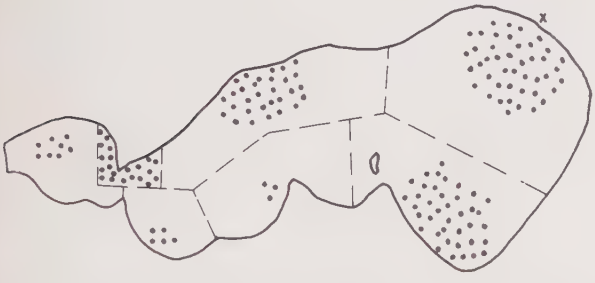

FIGURE 5. RECAPTURES OF TAGGED PIKE RELEASED AT THE HATCHERY.

FIGURE 6. RECAPTURES OF TAGGED PIKE RELEASED AT WITKE'S.

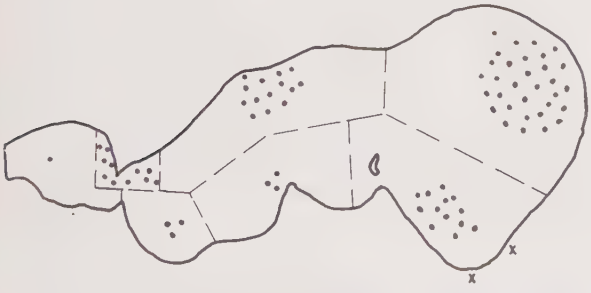

FIGURE 7. RECAPTURES OF TAGGED PIKE RELEASED AT THE STATE PARK AND AT SOUTH BAY.

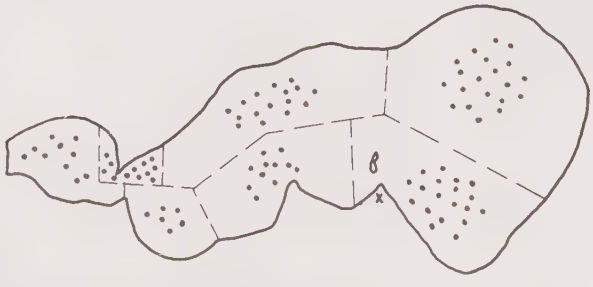

FIGURE 8. RECAPTURES OF TAGGED PIKE RELEASED AT BAYSIDE.

TABLE 1.--Numbers of tagged pike stocked at each point in Clear Lake, Iowa, October 1953, and numbers from each stocking caught in each area of the lake in 1954, compared to the numbers expected (in parentheses) if pike from all plantings distributed themselves in the same fashion

Area	Stocking location	Number stocked	I	II	III	IV	V	VI	VII	Total per location
III	Clausen's Cove--	50	1 (1.0)	3 (1.5)	----- (0.4)	5 (1.8)	----- (0.5)	------ (2.7)	1 (2.0)	10
IV	Rushes----------	608	28 (16.1)	27 (24.9)	9 (6.8)	30 (30.5)	9 (8.6)	32 (44.1)	29 (33.0)	164
IV	State Dock------	306	12 (8.1)	11 (12.5)	3 (3.4)	15 (15.2)	5 (4.3)	21 (22.0)	15 (16.5)	82
VI	Hatchery--------	704	8 (14.7)	21 (22.8)	5 (6.2)	30 (27.9)	3 (7.8)	44 (40.3)	39 (30.2)	150
VI	Witke's---------	253	4 (7.3)	14 (11.3)	1 (3.1)	11 (13.8)	3 (3.9)	26 (19.9)	15 (14.9)	74
VII	Bayside---------	379	10 (9.2)	12 (14.3)	6 (3.9)	16 (17.5)	11 (4.9)	20 (25.3)	19 (18.9)	94
VII	State Park and South Bay	330	1 (7.6)	11 (11.7)	3 (3.2)	14 (14.3)	3 (4.0)	32 (20.7)	13 (15.5)	77
	Totals per area		64	99	27	121	34	175	131	651
	Percentage per area		9.82	15.21	4.15	18.59	5.22	26.88	20.12	100

As the fish from the various plantings are not caught in the same proportions in various parts of the lake, it is interesting to determine where the differences occur. Table 1 compares the numbers caught to the numbers expected if the fish from each planting were distributed the same as those of all plantings together.

It is obvious that the pike do not remain in unusual proportions in the area in which they were planted. Slightly greater than expected catches of the Witke's and the Hatchery-stocked fish were taken in area VI, but all other stockings showed less than the expected catch in the area of stocking. The differences, however, are all so small as likely to be due to sampling variation (Chi-square = 3.0 with 6 degrees of freedom).

The places where the deviations from the expected are large, are the following:

1. The fish stocked in area IV were taken in more than expected numbers in area I, and possibly in less than expected numbers in areas VI and VII at the other end of the lake.
2. The fish stocked in area VI and at the State Park in area VII were taken in less than expected numbers in area I, and perhaps in more than expected numbers in areas VI and VII.

Summary

The results of this tagging experiment indicate that stocked northern pike move to all parts of Clear Lake within 6 months, even if stocked at one end. Although the movement is extensive and general, there is evidence that the fish stocked in some areas do not get to areas at the other end of the lake in quite the same abundance. A longer period of time between stocking and capture of the fish might eliminate even this slight difference in their distribution.

Acknowledgments

The authors express their appreciation to all who helped on this project, particularly Mr. and Mrs. James G. Erickson, who tagged the pike; Muhrl Lindquist, hatchery supervisor, who stocked the fish; Charles DiCostanzo, who aided in the field work; the Clear Lake Chamber of Commerce and all anglers who reported the tagged fish; and Conservation Officer Jack Stevens.

Literature Cited

Carlander, K. D.
1953. Use of gill nets in studying fish populations, Clear Lake, Iowa. Proc. Iowa Acad. Sci. 60: 621-625.

NEW ABSTRACTING SERVICE AVAILABLE FOR FISHERY WORKERS

The U. S. Fish and Wildlife Service has recently started the publication of Sport Fishery Abstracts, a new serial pamphlet that is designed to provide an abstracting service for those who are engaged in sport fishery research and management. The new publication will contain abstracts of current papers that are concerned primarily with sport fish and fishing problems. Abstracts of papers dealing with allied fields of specialization, such as limnology, ecology, oceanography, and natural history, will be included when the subject matter has application to the conservation of sport fish and the restoration of sport fishing.

Sport Fishery Abstracts will be available without charge, but distribution of the publication will be limited to those who have a direct interest in conservation activities.

A SIMPLE CHEMICAL TEST FOR ROTENONE IN WATER

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A COLOR TEST is being used at the Wyoming Game and Fish Research Laboratory at the University of Wyoming to give a quantitative estimate of rotenone in water. The simplicity of the method makes it practical for field use to determine the quantity of rotenone present in water during fish-poisoning operations, and to check on residual amounts of the toxicant before restocking the water. It may replace the use of live fish and live cars in many instances.

The method is based on a qualitative test for rotenone devised by Jones and Smith (1933) and later improved by Rogers and Calamari (1936). The reagents required are chloroform, 10 percent thymol in chloroform, and hydrochloric-nitric acid reagent (0.2 milliliter of concentrated nitric acid in 100 milliliters of concentrated hydrochloric acid). Both the thymol reagent and the hydrochloric-nitric acid reagent are quite stable. The acid reagent, however, must be stored in a bottle with an acid-resistant closure. The apparatus required consists of a 500-milliliter separatory funnel, 4 pipettes (a 5-, a 2-, and two 1-milliliter), a 500-milliliter graduated cylinder, a few test tubes, and a color chart. An optional piece of equipment is a rubber bulb or other safety pipette filter for pipetting the volatile reagents.

The procedure found most satisfactory at this laboratory consists of extraction of 500 milliliters of suspected water with 5 milliliters of chloroform in a separatory funnel. The chloroform-water mixture is shaken vigorously for at least a minute; then the chloroform is allowed to settle to the bottom of the container. After the chloroform layer is well formed, it is collected in a test tube. Care should be taken to get as little water as possible. A second extraction of the water remaining in the separatory funnel is then made with 2 milliliters of chloroform, and the chloroform layer is added to the previous collection in the test tube. One milliliter of the thymol reagent is then added to the chloroform and followed by 1 milliliter of the hydrochloric-nitric acid reagent. The tube is twirled carefully for 30 seconds to mix the reagents and is then set aside. The color will usually begin to develop in the chloroform layer within 5 minutes or less and will be complete within an hour. The color ranges from a tan (when no rotenone is present) to a yellow green, to a green blue (with increasingly higher concentration of rotenone). The amount of green color produced is an indication of the amount of rotenone present. The presence of any green may be considered as a positive test for rotenone. One must keep in mind that the test given by Rogers and Calamari (1936) is considered a qualitative test because its accuracy is not dependable enough for strict quantitative work; however, the use of a color chart yields a fair estimate of the amount of rotenone present.

The color chart used in this procedure was made up from the color produced by chemically pure rotenone in known

Contribution from the Federal Aid in Fisheries Restoration Act Project FW-3-4, Wyoming.

concentrations. The extraction method was used on 500-milliliter aliquots of distilled water containing 0.00, 0.01, 0.02, 0.03, 0.04, and 0.05 part per million of rotenone (equivalent to 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 part per million of a 5-percent rotenone carrier such as Noxfish, Chem-fish, or Cubé root). The colors produced were matched with nonfading water colors. Areas were painted on paper in the shape of test tubes, and the paper was laminated between plastic sheets. Laminating the card yields a water-resistant, soil-resistant, stable set of colors for laboratory or field use.[1/]

The method has been found satisfactory to determine the rotenone in water bearing Noxfish, Chem-fish, and powdered Cubé. The method has given good results in natural water up to 35,000 parts per million of total solids.

Literature Cited

Jones, H. A., and C. M. Smith.
1933. A color test for rotenone. Ind. Eng. Chem., Anal. Ed. 5: 75-76.

Rogers, H. D., and J. A. Calamari.
1936. Rotenone determination by colorimetric methods. Ind. Eng. Chem., Anal. Ed. 8: 135.

[1/] The Wyoming Game and Fish Commission has copies of this color chart available for free distribution. Requests should be addressed to the author.

THE CULTURE OF DAPHNIA

Because of the widespread interest that has been indicated in the culture of Daphnia as an excellent food for tropical fish of all varieties, Harvey A. Bender of Western Reserve University, Cleveland, Ohio, has recently summarized pertinent information.

"Daphnia," writes Mr. Bender, "are easily raised at home with the minimum of effort and the barest of facilities."

A wide-mouthed gallon container made of glass or porcelain--metallic vessels, especially those lined with zinc or lead, should be avoided--may be expected to supply the needs of the average aquarium. The container should be washed thoroughly and filled with pond water or with tap water that has been standing for 24 hours. It is then ready for inoculation with a number of Daphnia, which should be fed at once.

Daphnia should be fed at least once a week. When the culture becomes heavy with these little aquatic crustaceans, feeding them twice a week is recommended. Food may be provided by means of a yeast solution made from a 5-cent package of dry commercial yeast. The yeast solution may be made by shaking a small amount of the dry yeast into some water. A package of dry yeast should be sufficient for 4 or 5 feedings. Enough of the yeast solution should be used in a single feeding to turn the water slightly milky in color (15 to 20 drops used in a gallon container).

When the culture provides a plentiful supply of Daphnia, they should be fed to the fish in the aquarium. However, the stock in the culture should never be wholly depleted.

Loss of water may be compensated for by the addition of tap water that has stood overnight.

"Daphnia cultures may be kept almost anywhere," Mr. Bender concludes, "but apparently they do best in indirect light and in cool surroundings, such as basements and out-of-the-way corners."

A QUICK METHOD OF PREPARING PERMANENT FIN-RAY AND SPINE SECTIONS

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DETERMINATION OF AGE from cross sections of fin-rays and spines has been demonstrated for many species of fish, but the time required for preparation of permanent study sections has always been a serious objection to use of this technique. A procedure that has been developed for mounting cross sections of marginal fin-rays of white sturgeon (Acipenser transmontanus Richardson) is rapid and provides workable study sections.

Sections of dry fin-rays, 1 millimeter or less in thickness, are cut with a jeweler's handsaw that is equipped with a 70-tooth-per-inch blade. A high-speed power saw was tried, but apparently the heat generated made the entire section opaque. The sections are smoothed on one side with No. 180 silicon carbide paper and polished with well-worn silicon carbide paper or any very fine grit paper. Although it was discovered that sections placed on the fingertip for the grinding could be handled easily, some individuals may find it easier to use a double-surfaced adhesive for this purpose.

The polished side of the section is imbedded in a drop of a nitrocellulose cement on a cellulose acetate slide that measures 0.060 inch in thickness. More than 2 large sections per slide may cause the slide to warp, but the slide can be straightened easily by dipping it into hot water and then flattening. The slide is then inverted on the same paper used previously and ground and polished by hand. The thickness of the mounted sections can be worked down from about 0.060 to 0.010 inch in less than a minute. The proper thickness can be determined quickly with a microscope using transmitted light.

The same technique applied to sections of pectoral spines of white catfish (Ictalurus catus Linne) yields excellent results with a minimum of effort. Sections taken from 6-inch fish are held firmly in the glue so that no chipping occurs during the grinding. If the sections are taken from young fish, it may not be necessary to grind the lower side of the section before gluing it to the slide.

All materials are easily obtained and inexpensive. Any slow-drying model-airplane cement may be used for the work.

As none of the sections has shown any tendency to come loose after several months, no cover slip need be used for storage.

The requirements for a satisfactory method of preparing fin-ray and spine sections for study have been met in that the grinding and mounting take only a short time and the sections are free of mounting imperfections. With practise, 1 man can turn out about 10 slides per hour with 2 sections on each slide.

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